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APHID TRANSMISSION OF NONPERSISTENT PLANT VIRUSES WITH SPECIAL REFERENCE TO THE *BRASSICA NIGRA* VIRUS[1]

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INTRODUCTION

DURING THE past few decades the study of transmission of plant viruses by insects has become increasingly important. Of the various groups of insects which act as vectors, aphids are probably the most important, both in number of species which are vectors and in number of different viruses transmissible by them.

It is the intention of this paper to report on details of some recent experimental work on transmission of the *Brassica nigra* virus (Takahashi, 1949), and to review the knowledge to date concerning aphid transmission of nonpersistent viruses in order to present a hypothesis on the mode of transmission of this group of viruses by aphids. The first portion is concerned with unreported experimental work.

Materials and Methods

The *Brassica nigra* virus was originally obtained from Dr. W. N. Takahashi, and has been maintained in *Brassica juncea* Coss. in this laboratory for experimental purposes. Test plants were *B. juncea* seedlings, unless otherwise noted. The vector principally used was the green peach aphid, *Myzus persicae* (Sulzer), with some trials also including the turnip aphid (= the false cabbage aphid), *Rhopalosiphum pseudobrassicae* (Davis). Noninfective colonies were maintained on healthy *B. juncea* plants. Insects were transferred with a camel's hair brush, and the acquisition feedings, unless otherwise noted, were watched and timed with a stop watch. The plants were fumigated after the test-feeding period before being placed in the greenhouse for incubation. The greenhouses were routinely sprayed to prevent aphid buildup. More detailed descriptions of methods are given in connection with specific experiments.

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Results

Effect of Forced Interruption of Acquisition Feeding on Transmission. When using limited watched and timed acquisition feedings during transmission trials with nonpersistent viruses, the insects very frequently are picked off the plant at the end of a specified time and consequently the acquisition feeding is unnaturally terminated. The same is true of test feedings. The effects of this on vector efficiency were first investigated by Bradley (1952). He concluded that the probability of obtaining an infective aphid was less under conditions of forced interruption of the acquisition feeding when compared with aphids allowed to terminate the penetration naturally. However, forced interruption of the test feeding did not seem to affect the efficiency of transmission. Further data were collected on this point through use of the green peach aphid and the *Brassica nigra* virus.

A group of aphids was fasted and divided into four lots. Individuals of lot 1 were allowed a normally terminated acquisition feeding on a virus source and then were transferred to a test plant and allowed a normally terminated test feeding. Aphids of lot 2 were allowed a normally terminated acquisition feeding, but the test feeding was artificially interrupted at the end of 15 seconds. Those of lot 3 were forcibly interrupted during the acquisition feeding at the end of 15 seconds, but allowed a normally terminated test feeding. The individuals of lot 4 were artificially interrupted after a 15-second acquisition-feeding period and also after a test-feeding period of 15 seconds.

In the factorial experiment of nine replications, five plants per treatment, the time values for the various feeding periods were: lot 1—acquisition feeding (excluding one value each of 75 seconds and 319 seconds) 16.63 $\pm$ 4.35 seconds, test feeding (excluding one value each of 138, 129, 86, and 65 seconds) 10.09 $\pm$ 2.13 seconds; lot 2—acquisition feeding 16.8 $\pm$ 5.59 seconds, test feeding 15.16 $\pm$ 1.37 seconds; lot 3—acquisition feeding 15.0 $\pm$ 0.0 seconds, test feeding (excluding one value each of 226, 156, and 142 seconds) 18.40 $\pm$ 12.24 seconds; and lot 4—acquisition feeding 15.0 $\pm$ 0.0 seconds, test feeding 15.0 $\pm$ 0.0 seconds. The preliminary fasting time was 1.53 $\pm$ 0.33 hours, and the temperature during the replications was 23.26 $\pm$ 0.3°C.

The results of the trials are given in table 1. It is evident that an acquisition feeding allowed to terminate normally will increase the probability of producing an infective insect (36 infections compared with 20 infections, $\chi^2 = 5.56$, $P = < 0.02$). However, whether the test feeding was terminated artificially, or was allowed to proceed to a normal conclusion, made little difference in the effectiveness of the feeding so far as transmission was concerned (26 infections compared with 30 infections, $\chi^2 = 0.35$, $P = < 0.50$).

In various transmission trials using the green peach aphid and the *Brassica nigra* virus, with *B. juncea* as the host plant, experience and observation have shown that during an initial puncture a normal interruption occurs approximately 15 seconds after feeding begins. Consequently, the use of 15 seconds as a standard acquisition-feeding period is probably not too unnatural, for while some reduction in potential infectivity occurs, many

of the 15-second, time-watched feedings have terminated naturally, and those which are not terminated naturally will be distributed at random throughout the various replications. However, the influence of artificially interrupting acquisition feedings on resulting vector efficiency must be considered when projecting laboratory results into the speculative realm of field application. The influence of artificially terminating penetrations may also explain the drop in efficiency during acquisition threshold periods trials at the longer intervals of 25 and 30 seconds, for this is a range where artificial interruption might have a greater probability of occurring.

TABLE 1

RESULTS* OF TRIALS TO DETERMINE THE INFLUENCE OF ARTIFICIALLY TERMINATING AN ACQUISITION- OR A TEST-FEEDING PERIOD ON THE EFFICIENCY OF TRANSMISSION OF THE *BRASSICA NIGRA* VIRUS TO *B. JUNCEA* SEEDLINGS BY FASTED *MYZUS PERSICAE* (SULZ.)

Acquisition feeding normally terminated		Acquisition feeding artificially terminated	
Test feeding normally terminated	Test feeding artificially terminated	Test feeding normally terminated	Test feeding artificially terminated
17/45	19/45	9/45	11/45

* In ratios listed, numerator is number of plants infected; denominator, number inoculated.
The preliminary fasting period was 1 to 2 hours long.

Effects of Fasting on Transmission. The acquisition, inoculation, and transmission threshold periods were evaluated previously for the green peach aphid transmitting the *Brassica nigra* virus using preliminarily fasted insects as a routine procedure (Sylvester, 1950*b*). To date no report has been made concerning the effects of fasting on transmission, although the benefits of fasting prior to short acquisition feedings on transmission on nonpersistent aphid-borne viruses in general have been well established (Watson, 1938; Watson and Roberts, 1939). The effects of post-acquisition feeding fasting are not so well known. There are enough published data, however, (Hoggan, 1933; Watson, 1938) to indicate that virus charge is lost by an infective aphid soon after acquisition whether it feeds or fasts. Further experimental work is needed to determine the effect of specific variations in these phenomena.

In tests the green peach and turnip aphids were compared by using 30 replications, one plant per treatment. In each replication the virus source and aphid colony were constant. The fasting intervals in both the pre- and post-acquisition feeding trials were 0, 5, 10, 15, 60, and 240 minutes.

In trials on the effects of pre-acquisition fasting on transmission efficiency, an acquisition feeding of 19.41 $\pm$ 2.58 seconds was used for the green peach aphid, and 23.30 $\pm$ 2.95 seconds for the turnip aphid. The intended test feeding was 60 minutes; the actual values were 69.29 $\pm$ 16.12 and 69.33 $\pm$

16.26 minutes for the green peach and the turnip aphids respectively. The values are based on records of 210 individuals of each species.

In the pre-acquisition fasting tests the green peach aphid was a more efficient vector than the turnip aphid (108 compared with 24 transmissions). The effects (table 2) of preliminary fasting were most pronounced at the 4-hour interval (20 infections compared with 11 infections, adjusted $\chi^2 = 8.2$, $P = < .01$). However, with the green peach aphid a response trend might have been indicated at the 5-minute interval. The turnip aphid was inefficient regardless of fasting period used, and the effects of preliminary fasting were not significant until 4 hours (nine infections compared with

TABLE 2

RESULTS* OF TRIALS TO DETERMINE EFFECTS OF PRE- AND POST-ACQUISITION FEEDING FASTING ON EFFICIENCY OF TRANSMISSION OF THE *BRASSICA NIGRA* VIRUS TO *B. JUNCEA* SEEDLINGS BY SINGLE GREEN PEACH AND TURNIP APHID APTERAE

Species	Pre-acquisition fasting period in minutes							Total
	0	5	10	15	30	60	240	
Green peach aphid	11	15	14	16	17	15	20	108
Turnip aphid	2	1	2	1	3	6	9	24
Species	**Post-acquisition fasting period in minutes**							**Total**
	0	5	10	15	30	60	240	
Green peach aphid	15	0	2	2	1	0	0	20
Turnip aphid	2	0	0	1	0	0	0	3

* Numbers in columns are number of plants infected out of 30 inoculated. Acquisition feedings were approximately 20 to 25 seconds; test feedings, 1 hour.

two infections, adjusted $\chi^2 = 4.12$, $P = < 0.5$), although a strong tendency for increased efficiency occurred at the 1-hour interval. The results are similar to those obtained with some other viruses (Watson, 1938, 1946; Watson and Roberts, 1939; Kassanis, 1941; Kvíčala, 1947; Sylvester, 1949*b*, 1952).

In trials on the effects of post-acquisition fasting on vector efficiency (table 2), a variable preliminary fasting period was used, which had a value of 89.83 $\pm$ 33.32 minutes, and 89.19 $\pm$ 29.34 minutes for the green peach and turnip aphids, respectively. An acquisition feeding of 18.85 $\pm$ 2.60 seconds was used for the green peach aphid, and 23.95 $\pm$ 2.36 seconds for the false cabbage aphid. A test-feeding period of approximately 60 minutes was intended, and the actual values were 69.43 $\pm$ 19.33 and 69.27 $\pm$ 18.24 minutes for the green peach and turnip aphids respectively. The figures are from records on 210 individuals of each species.

In tests on effects of post-acquisition fasting the green peach aphid again was the better vector (table 2). Five minutes' starvation decreased infectivity, after which little difference existed between this and the other intervals tested. The inefficiency of the turnip aphid made the data of little comparative value. However, no efficiency increase was noted in the fasted

insects. Presumably a longer preliminary fasting would have increased the initial infectivity.

Kassanis (1941) reported that loss of virus charge by infective aphids was slower when the temperature was lowered. For confirmatory purposes tests with the green peach aphid were done in the present study, using 11 replications, five plants per treatment for each variable. Comparisons were made under two temperature conditions, room (22°C) and refrigerator (5°C), using two preliminary fasting levels (1 hour and 4 hours) and two post-acquisition fasting levels (30 minutes and 1 hour). A nonfasted check at room temperature was also included.

TABLE 3

RESULTS* OF TRIALS TO DETERMINE EFFECTS OF LOW TEMPERATURE ON INFLUENCE OF PRE- AND POST-ACQUISITION FASTING PERIODS ON TRANSMISSION OF THE *BRASSICA NIGRA* VIRUS TO *B. JUNCEA* SEEDLINGS BY SINGLE GREEN PEACH APHID APTERAE

Temperature								
Room (22° C)					Refrigerator (5–6° C)			
Preliminary fasting (*in hours*)			Post-acquisition fasting (*in hours*)		Preliminary fasting (*in hours*)		Post-acquisition fasting (*in hours*)	
0	1	4	0.5	1	1	4	0.5	1
2/55	5/55	14/55	1/55	0/55	6/55	7/55	8/55	2/55

* In ratios listed, numerator is number of plants infected; denominator, number inoculated. Acquisition- and test-feeding periods were approximately 15 seconds and 1 hour respectively.

The mean (range) acquisition feeding was 14.8 (11 to 15) seconds based on the 495 individuals used in the trials. The mean (range) test feeding, based on 99 groups of five aphids, was 60.9 (44 to 76) minutes. The mean (range) for the preliminary fasting period used in connection with the post-acquisition fasting trials was 67.4 (57 to 84) minutes based on 44 groups of five insects.

There was an apparent tendency for refrigerated aphids to be more restless. This could have influenced the results, since uncoöperative insects would have an additional, short, room-temperature fasting while the series of acquisition feedings was being completed. Likewise, insects would be more likely to wander off test plants. However, fasting in the refrigerator, compared with room temperature, did not cause abnormal restlessness, since the time required to feed five aphids was approximately equal regardless of prior treatment. The mean (range) for completion times was 6.3 (2 to 11) minutes for room-fasted insects compared with 7.1 (3 to 16) for refrigerator-fasted aphids. Aphids missing from plants at the end of the test feedings were 15 out of 275 for room-fasted and 18 out of 220 for refrigerator-fasted insects.

Comparable transmission was obtained when preliminary fasting for 1 hour was at either 22°C or 5°C (table 3). After 4 hours of preliminary

fasting, insects at 22°C were more efficient than those fasted at 5°C. Loss of infectivity during a post-acquisition fasting period was slower at 5°C. At 22°C nearly all virus charge disappeared when infective aphids were fasted for 30 minutes. At the end of an hour no infective aphids were found. In contrast, when infective insects were fasted at 5°C, infectivity was maintained at a high level for 30 minutes, and some infective aphids were found after an hour. Kassanis (1941) obtained comparable results with tobacco etch virus.

Effects of Multiple Acquisition Feedings on Transmission. The influence of multiple stylet penetrations by insect vectors on transmission of plant viruses has lacked extensive investigation. However, it has been established in connection with both a persistent and nonpersistent virus that the prob-

TABLE 4

RESULTS* OF TRIALS IN THE TRANSMISSION OF *BRASSICA NIGRA* VIRUS TO *B. JUNCEA* SEEDLINGS BY FASTED SINGLE GREEN PEACH APHID APTERAE TO DETERMINE THE INFLUENCE OF MULTIPLE SEPARATE 15-SECOND ACQUISITION FEEDINGS ON VECTOR EFFICIENCY

Number of sequential separate 15-second acquisition feedings									
1	2	3	4	5	6	7	8	9	10
11/30	11/30	14/30	14/30	18/30	23/30	17/30	18/30	17/30	18/30

* In ratios listed, numerator is number of plants infected; denominator, number inoculated.
The sequential acquisition feedings ranged from one 15-second acquisition feeding up to and including ten 15-second acquisition feedings. The test feeding period was approximately 1 hour.

ability of obtaining successful inoculation can be increased by forcing an infective aphid to make a series of separate stylet penetrations on a test plant. Similar effects can be achieved by increasing the number of insects. It also can be shown that with nonpersistent viruses the chances of individual aphids becoming infective increase, within limits, as the number of separate acquisition feedings increase (Sylvester, 1950*a*). The following experiments give additional support for these statements.

Thirty replications of the following experiment were made. A single green peach aphid aptera, selected from a fasted population, was given one of the following acquisition-feeding sequences: one 15-second acquisition feeding, two separate 15-second acquisition feedings, et cetera, up to and including 10 separate 15-second acquisition feedings. Following the acquisition feedings, each aphid was placed on a healthy *B. juncea* seedling, and allowed a test-feeding period of approximately 1 hour. The experiment was replicated 30 times for each interval, and the order of use of the acquisition-feeding sequence was randomized at each trial.

Based on records of 300 individuals, the values for feeding and fasting times were: pre-acquisition fasting, 1.74 ± 0.33 hour; each separate acquisition feeding 14.86 ± 0.3 seconds, test feeding, 1.18 ± 0.19 hour. Time to complete the various acquisition-feeding sequences ranged from 0.25 ± 0.0 minutes to complete one acquisition feeding to 9.30 ± 2.20 minutes to complete 10 separate acquisition feedings.

The results (table 4) indicate that if the separate acquisition feedings were four or less, little increase in infectivity occurred when multiple acquisition feedings were used instead of a single-acquisition feeding. If five or more separate acquisition feedings were used there was a tendency for increased vector efficiency (17 out of 30 infections compared with 11 out of 30 infections, adjusted $\chi^2 = 1.67$, $P = .20$ approximately). In the trials six was the best number of acquisition feedings (23 out of 30 compared with 11 out of 30, adjusted $\chi^2 = 8.84$, $P = <0.01$), but there was a doubtful tendency for real differences to exist between any of the multiple acquisition-feeding sequences between 5 and 10 (23/30 compared with 17/30, adjusted $\chi^2 = 1.95$, $P = >.10$).

Assuming that the chance for virus pickup is independent of previous feeding activity and that the charge acquired during any feeding is not lost, but accumulated during any subsequent acquisition feeding, the probability of obtaining infective insects should follow the binominal theory of distribution. If P represents the probability of obtaining an infective insect with one acquisition feeding, and q represents the probability of not obtaining an infective individual with one feeding, then p, the probability of obtaining an infective insect with x feedings would be $= 1 - q^x$. Using the value of $P = 0.37$ (11/30) as the probability of obtaining an infective aphid with one feeding, the p value for one to 10 separate feedings would be: 0.37, 0.063, 0.742, 0.837, 0.897, 0.935, 0.969, 0.974, 0.984, and 0.990, respectively. It is obvious (table 3) that the acquisition of virus during sequential separate acquisition feedings does not follow a binominal distribution. Presumably the insects may acquire and/or disperse virus during each penetration, and accumulative charge is not reflected in subsequent test feedings. However, since dispersal is probably incomplete, a slight increase in probability occurs in favor of the exchange resulting in slightly increased infectivity.

Serial Transmission. Serial transmission of nonpersistent viruses by aphids has been well established (Watson, 1938; Kassanis, 1941; Kvíčala, 1948*a*, 1949*b*; Severin and Tompkins, 1950*a*; Sylvester, 1950*a*). It is one test of vector potency, and as such possibly can be used to measure the level of virus charge acquired under various feeding conditions. The following tests on serial transmission of the *Brassica nigra* virus by single green peach aphids support previous conclusions based on similar experiments (Sylvester, 1950*a*).

Three acquisition-feeding variations were used: (A) one 15-second acquisition feeding; (B) five separate successive 15-second acquisition feedings; and (C) 10 separate successive 15-second acquisition feedings. Following the acquisition-feeding sequence, individual aphids were moved to healthy plants for a 15-second test-feeding period, after which they were transferred to a second plant for a similar feeding. This procedure was repeated until 20 seedlings had been fed on, each for 15 seconds. The experiment was repeated 30 times. The aphids and test plants were selected at random from uniform populations, and the sequence of use of the acquisition-feeding variations (A, B, or C) was randomized. Aphids were fasted before beginning the acquisition feedings. Additional data concerning the values of the fasting periods, acquisition feedings, test feedings, and completion times are as follows: preliminary fasting (A) 1.93 $\pm$ 1.23 hours, (B) 2.18 $\pm$ 1.29

TABLE 5

DISTRIBUTION OF INFECTIONS* OF *BRASSICA NIGRA* VIRUS WHEN SINGLE GREEN PEACH APHIDS WERE FED CONSECUTIVELY ON 20 HEALTHY *B. JUNCEA* SEEDLINGS

Successive test feeding plant number																				Frequency†
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Series A, one 15-second acquisition feeding:																				
+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	1
+	+	+	−	+	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	1
+	+	+	−	+	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	1
+	−	−	+	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	1
−	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	1
−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	1
−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	1
−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	1
−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−	1
−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	1
−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	1
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	1
Series B, five 15-second acquisition feedings:																				
+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	3
+	+	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	1
+	0	−	−	+	−	−	−	−	−	−	+	−	−	−	−	−	−	+	−	1
+	−	−	+	+	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	1
+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	1
−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	1
−	+	+	+	+	+	−	−	+	−	−	−	−	−	−	−	−	−	−	+	1
−	+	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	1
−	+	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−	1
−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	2
−	−	+	+	−	−	−	−	−	−	+	−	−	−	−	+	−	−	+	−	1
−	−	+	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	1
−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	1
−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	2
−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	1
−	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	1
−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	1
Series C, ten 15-second acquisition feedings:																				
+	−	+	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	+	1
+	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	1
+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	1
+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	1
+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	1
−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	1
−	+	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	1
−	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	1
−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	1
−	−	+	−	−	−	−	+	−	+	−	−	−	+	−	−	−	−	−	−	1
−	−	−	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	1
−	−	−	+	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	1
−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	1
−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	1
−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	1
−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	1
−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	1
−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	+	1
−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	1
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	1
−	−	−	−	−	−	−	0	−	−	−	−	−	−	−	−	−	−	−	+	1

* Plus sign (+) indicates production of disease, minus sign (−) shows that no disease resulted, and the zero sign (0) indicates that plant died.

The insects were fasted and then given (A) 1, (B) 5, or (C) 10 separate acquisition feedings. The subsequent test feedings were approximately 15 seconds each.

† Frequency refers to number of times distribution occurred.

hours, (C) 2.09 ± 1.24 hours; acquisition feeding (A) 15.0 ± 0.0 seconds, (B) 14.59 ± 1.94 seconds, (C) 14.80 ± 0.84 seconds; time to complete the sequence of acquisition feedings (A) 0.25 ± 0.0 minutes, (B) 3.13 ± 0.76 minutes, (C) 7.43 ± 2.91 minutes; test feedings (A) 14.74 ± 1.07 seconds, (B) 14.64 ± 1.24 seconds, (C) 14.69 ± 0.91 seconds; time to complete sequence of test feedings (A) 21.93 ± 8.98 minutes, (B) 22.70 ± 6.48 minutes, (C) 24.63 ± 6.16 minutes.

The results (tables 5, 6, and 7) were similar to those obtained in the serial transmission of beet mosaic virus (Sylvester, 1950*a*), where it was shown that dispersal of virus charge by individual aphids was somewhat at random, and that multiple-acquisition feedings, while insuring more infective aphids in a population, did not effectually increase the infectivity level of individuals.

Access Time. At times in experimental work it is neither convenient nor feasible to watch individual aphids during specific acquisition feedings. This is particularly true when acquisition feedings exceed 30 seconds. It is also difficult to get individual insects to maintain single initial penetrations consistently for more than 30 seconds. Numerous reports (Watson, 1938; Watson and Roberts, 1939; Kassanis, 1941; Kvíčala, 1948*a*, 1949*a*; Hamlyn, 1953) state that with nonpersistent viruses vector efficiency decreases as the length of the acquisition feeding increases. However, when large experiments are designed, not involving critical comparative vector-feeding studies, which necessitate adequate standardization of the acquisition-feeding period, it would be helpful to use an access period which would insure near maximum vector efficiency for any specific vector-virus combination. The term access period refers to a period of time during which a vector has access to a virus source, but during which feeding for the entire allotted time may or may not occur.

To obtain data on access periods, green peach aphids, fasted for 4 hours, were transferred in lots of 50 to one leaf of a virus source. Five individuals were allowed a watched and timed 15-second acquisition feeding, and then were transferred singly to five healthy *B. juncea* seedlings. The remaining individuals were allowed to stay on the virus source for periods of 5, 15, 30, 60, and 240 minutes. At the end of each interval, five feeding aphids were selected and transferred singly to healthy test plants. During the test feeding the plants were caged with glass vials (32 × 90 mm) and the insects allowed to feed overnight (24-hour test feeding), after which the cages were removed and plants fumigated with nicotine. The experiment was repeated eight times.

The results (table 8) indicated that an access time of 15 minutes could be used before any decrease in vector efficiency occurred when compared with a measured 15-second acquisition feeding (10/40 infections compared with 12/40). The access time of 5 minutes was the most favorable of all tested (20/40 infections), and increasing the access time beyond 15 minutes was detrimental.

Three factors might explain the tendency for the 5-minute access time to be superior to the 15-second acquisition feeding (20/40 infections compared with 12/40, $\chi^2 = 3.2$, $P = < .10$). First, individual aphids had oppor-

tunity to feed for more than 15 seconds, which might be advantageous since it has been shown that 20-second acquisition feedings are sometimes better than 15 seconds (Sylvester, 1950*b*). Secondly, individuals would have opportunity to make several stylet penetrations. Thirdly, several of the short feedings might be naturally interrupted. This would increase the probability of obtaining an infective individual (Bradley, 1952). The same reasoning

TABLE 6

SUMMARIZATION* OF TABLE 5 (DISTRIBUTION OF INFECTIONS OF *BRASSICA NIGRA* VIRUS WHEN SINGLE GREEN PEACH APHIDS WERE FED CONSECUTIVELY ON 20 HEALTHY *B. JUNCEA* SEEDLINGS)

Series	Successive plant number																				Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
A.....	4	2	4	3	5	1	0	1	4	1	0	0	1	1	1	0	2	1	0	0	31
B.....	7	5†	6	5	5	4	2	2	1	2	1	2	1	0	0	2	1	1	2	1	50
C.....	5	3	4	2	2	1	4	4†	4	4	2	2	1	1	0	0	1	1	4	2	47
Total....	16	10	14	10	12	6	6	7	9	7	3	4	3	2	1	2	4	3	6	3	128

* Numbers in columns are number of plants positive out of 30 inoculated.
The insects were fasted, then given (A) 1, (B) 5, or (C) 10 separate 15-second acquisition feedings. Subsequent serial test feedings were approximately 15 seconds each.
† In these instances one of the test plants died.

TABLE 7

SUMMARIZATION OF RESULTS IN TABLES 5 AND 6 (SERIAL TRANSMISSION OF *BRASSICA NIGRA* VIRUS TO *B. JUNCEA* SEEDLINGS BY GREEN PEACH APHID APTERAE*)

Series	Number of plants infected	Number of aphids infective	Mean number of plants infected per aphid	Frequency with which a single aphid infected 1, 2, 3, 4, 5, 6, or 7 plants						
				Plants						
				1	2	3	4	5	6	7
A.....	31/600†	12/30‡	2.58	6	1	2	1	0	1	1
B.....	50/599	21/30	2.38	11	2	2	3	2	0	1
C.....	47/599	21/30	2.23	6	7	6	1	1	0	0
			Total.....	23	10	10	5	3	1	2

* Insects were fasted, then given (A) 1, (B) 5, or (C) 10 separate 15-second acquisition feedings. Subsequent serial test feedings were approximately 15 seconds each.
† In ratios listed, numerator is number of plants infected; denominator, number inoculated.
‡ In ratios listed, numerator is number of aphids infective; denominator, number tested.

would apply to the 15-minute access time, except that the detrimental effects of prolonging acquisition feeding mitigated the beneficial effects of multiple stylet penetrations and optimum length of individual feedings. In access time beyond 15 minutes, the benefits to vector efficiency accruing from: 1) preliminary fasting; 2) multiple stylet penetrations; 3) natural stylet withdrawals; and 4) optimum length of individual feedings were obliterated by the deleterious effects of prolonged, continuous acquisition feeding. This phenomenon is somewhat characteristic of the transmission of nonpersistent viruses by aphids.

Vector Activity During Access Period. To evaluate more concretely the factors of multiple stylet penetration and duration of individual punctures which occur during a 5-minute access period, records were kept on individual aphids feeding on an infected *B. juncea* plant. The insects were fasted for 40 minutes to 5 hours, and then were placed on a diseased leaf and watched for 5 minutes. Records were kept of time spent in feeding and the number and duration of each stylet penetration. Based on limited trials (table 9) aphids during a 5-minute access period made on the average three or four punctures, the majority of which were longer than 15 seconds. However,

TABLE 8

RESULTS* OF TRIALS TO DETERMINE THE INFLUENCE OF VARIOUS ACCESS PERIODS† ON A VIRUS SOURCE, ON THE EFFICIENCY OF TRANSMISSION OF THE *BRASSICA NIGRA* VIRUS TO *B. JUNCEA* SEEDLINGS BY SINGLE FASTED GREEN PEACH APHID APTERAE

Replication	15-second feeding	Access period in minutes					Total
		5	15	30	60	240	
A	2	4	2	0	0	0	8
B	2	1	1	1	0	0	5
C	2	3	1	1	0	0	7
D	1	4	3	0	0	0	8
E	1	1	2	0	0	1	5
F	2	2	0	0	0	0	4
G	1	1	0	0	0	0	2
H	1	4	1	1	0	0	7
Total	12	20	10	3	0	1	46

* Numbers in columns are number of plants infected out of 5 inoculated.
The 15-second acquisition-feeding period was a measured feeding and was used as a standard for comparison. The test-feeding period was overnight.
† Access period is period of time during which a vector has access to a virus source, but may or may not be feeding for any or all of the period.

it is questionable whether or not these data justify the conclusion that they adequately explain the higher level of infectivity obtained in the 5-minute access period over a watched 15-second acquisition feeding (table 8). This is especially true when the data on multiple-puncture effects are considered (table 4), for in those tests it was shown that approximately five punctures were needed to increase infectivity. Other results (Sylvester, 1950*b*) also have indicated that a single acquisition feeding beyond 20 seconds did not necessarily increase infectivity; in fact, a drop in infectivity sometimes occurred after a 30-second acquisition feeding. Presumably some of the gain in efficiency was due to natural termination of the penetration, but since only feeding aphids were taken for testing at the end of the specified access time intervals, the last penetration was not naturally terminated. Of interest also is the fact that the 5-minute access period gave the most variable results (table 8). Much more uniformity occurred in the other periods, when comparisons were justified. The high variability between replications suggests that an interaction of variable consequence grading from favorable to unfavorable was especially important in the 5-minute trials. The interaction would include duration and repetition of stylet penetration.

Comparative Virus Loss During Access Time and Fasting Time. Numerous data have been collected on loss of nonpersistent viruses by both feeding and fasting insects (Watson, 1938, 1946; Kassanis, 1941; Kvíčala, 1948*a*, 1949*a*; Hamlyn, 1953). Some of these data have been comparative, some have not. In determining the loss of virus charge by feeding vectors, two approaches are commonly used. One is to extend the acquisition feeding for given intervals, and then test the insects on healthy plants, such as was done in the previous experiment, while the other is to use a uniform acquisition-feeding period or access time, and then remove the insects to a

TABLE 9

RECORDS OF MOVING AND FEEDING ACTIVITIES OF STARVED SINGLE GREEN PEACH APTERAE WHEN PLACED UPON A *BRASSICA JUNCEA* PLANT, INFECTED WITH *BRASSICA NIGRA* VIRUS, FOR AN ACCESS PERIOD OF 5 MINUTES

Aphid	Number of stylet penetrations made	Time, in seconds, spent at each stylet penetration						Mean number of seconds spent in wandering between penetrations
		Penetration number						
		1	2	3	4	5	6	
1	2	15.0	241.0*	....	....	....	....	22.0
2	2	40.0	235.0	....	....	....	....	12.5
3	2	30.0	225.0	....	....	....	....	22.5
4	3	12.0	70.0	120.0	....	....	....	32.6
5	3	21.0	26.0	176.0	....	....	....	25.6
6	3	175.0	9.0	41.5	....	....	....	24.8
7	3	21.0	21.0	163(w)†	....	....	....	23.8
8	3	24.0	148.0	74.5	....	....	....	17.8
9	4	20.0	12.0	168.0	22.0(†)	....	....	17.6
10	4	26.0	13.5	16.0	108.0	....	....	35.8
11	4	20.0	26.0	8.5	143.0	....	....	25.9
12	4	11.0	20.0	43.5	149.0	....	....	24.4
13	4	22.0	47.0	10.0	146.5	....	....	18.6
14	5	19.5	14.0	23.5	115.0	24.0	....	20.4
15	6	10.5	26.0	23.0	30.0	20.0	9.0	30.3

* Where no code letter follows the last figure, the insect was feeding when the 5-minute access period elapsed, and the insect was subsequently removed.

† Code letter (w) indicates that the insect was wandering when the 5-minute access period elapsed, and the insect was subsequently removed.

healthy plant, and from that plant transfer them to a second healthy plant at the desired intervals. Both methods have their disadvantages. If the insects are allowed to remain for the entire access time on the virus source plant, then the virus charge may be lost and reacquired several times during the experimental period (Bradley, 1953). On the other hand, if the insects are removed to a test plant for the variable feeding intervals, and then moved to a second healthy plant for final reading, the loss of virus is somewhat dependent on the feeding activities on the first plant. For example, the number of punctures effected while on the first plant will influence final vector infectivity. Loss of virus due to feeding activities does not occur in trials designed to determine virus loss during periods of post-acquisition fasting.

The first series of replications was designed to measure again the effects of prolonging the access time on infectivity of the green peach aphid. The

insects were fasted for 1.04 ± 0.04 hour, and then placed on a diseased leaf of a *B. juncea* plant infected with *Brassica nigra* virus. The insects were removed in lots of five at the end of 5, 15, and 30 minutes, and 1, 4, 8, and 24 hours, and tested singly on healthy mustard seedlings, using a test feeding of 1.00 ± 0.25 hour. Eight replications of the above procedure were done. The temperature during the experiment was 19.79 ± 3.5°C. The results of the trials are given in table 10. From the results it is again evident that loss of infectivity is noticeable after an access time of 15 minutes (35/40 compared with 21/39, adjusted $\chi^2 = 9.43$, $P = < 0.01$), and approximately 80

TABLE 10

RESULTS* OF TRIALS TO DETERMINE INFLUENCE OF LENGTH OF ACCESS TIME ON A VIRUS SOURCE ON INFECTIVITY OF GREEN PEACH APHIDS. VIRUS USED WAS *BRASSICA NIGRA* AND TEST PLANTS WERE *B. JUNCEA* SEEDLINGS

Length of access time						
5 minutes	15 minutes	30 minutes	1 hour	4 hours	8 hours	24 hours
35/40	21/39	17/40	3/40	1/40	0/40	0/40

* In ratios listed, numerator is number of plants infected; denominator, number inoculated.

Insects were given a preliminary fasting and then placed on a diseased plant for the designated time intervals, after which they were placed singly on test plants for a period of approximately 0.5 hour.

per cent loss of original infectivity occurred when the insects were allowed to feed for an hour. An occasional individual was infective after a 4-hour access period. Insects allowed access to a virus source for 8 or 24 hours were not infective. Watson (1946) reported in access-time trials with *M. persicae* and the beet mosaic virus that there was a rise in infectivity after an initial decline, and at the end of 24 hours of access time the insects were approximately equal in infectivity to those which had been fed for 5 minutes. There is nothing in the present work which would indicate the reason for the discrepancy between the reported results of Watson and those obtained in the present work, unless it was due to the viruses concerned.

The second group of experiments was done to compare the relative loss of infectivity in aphids which, after an access period of 5 minutes on a virus source, were fed on a healthy plant for specified intervals to a group which was fasted for the same intervals. A group of approximately 100 apterous green peach aphids was fasted in a vial for 70.0 ± 17.32 minutes. The aphids were then transferred as a group to a *B. juncea* leaf infected with the *Brassica nigra* virus, and allowed access to the virus for a period of 5 minutes, after which time they were removed. Approximately one half the sample was immediately placed on the leaf of a healthy mustard plant, while the other half was returned to the glass vial. At the end of 5, 15, 30, 60, 120, and 180 minutes samples of five insects from each group and one interval were placed singly on each of five *B. juncea* seedlings for a test feeding of approximately 0.5 hour. The experiment was replicated three times, given a total of 15 insects for each group and interval. The tempera-

ture during the experiment was 23.85 ± 1.06°C. The results of the trials are given in table 11. Infective insects retained virus longer during a fasting period than during a comparable feeding period on a healthy plant. The insects showed loss of infectivity during the first 5-minute access period on the healthy plant (9/10 compared with 4/15, adjusted $\chi^2 = 7.26$, $P = < 0.01$). This loss was probably due to multiple-feeding activities since most insects make three or four punctures before settling down (table 9), and the greatest loss of virus by infective insects occurs during the first few penetrations effected following an acquisition feeding (table 5). At the end of 30-minute access time on a healthy plant most of the insects had evidently lost their infectivity, for no insects were found to be infective at the 1-, 2-, or 3-hour intervals.

TABLE 11

RESULTS* OF TRIALS TO DETERMINE LOSS OF INFECTIVITY BY APHIDS WHICH WERE FED AFTER AN ACCESS FEEDING OF 5 MINUTES AS COMPARED WITH THOSE WHICH WERE FASTED. VECTOR USED WAS THE GREEN PEACH APHID; VIRUS WAS THE *BRASSICA NIGRA* VIRUS

Treatment	Time						
	0	5 minutes	15 minutes	30 minutes	1 hour	2 hours	3 hours
Fed	9/10	4/15	3/15	1/15	0/15	0/15	0/15
Fasted	....	11/15	8/15	4/15	8/15	5/15	1/15

* In ratios listed, numerator is number of plants infected; denominator, number inoculated.
Preliminary fasting period was approximately 1 hour, the test feeding on healthy *B. juncea* seedlings approximately 0.5 hour. The fed aphids were placed on a healthy *B. juncea* leaf for the desired interval before testing, the fasted aphids were placed in a glass vial during the intervals used before testing.

The insects which were fasted instead of fed during the post-acquisition tests were found to retain virus for 3 hours. However, there appeared to be a gradual loss of virus, the evidence of which was possibly reflected during the 5-minute intervals. Most of the infectivity was gone at the end of the third hour of post-acquisition fasting. The results of previous trials (table 2) indicate almost complete loss of virus from the insects during the first 15 minutes of post-acquisition fasting. The main difference between the two trials is that the aphids of table 2 were given a limited acquisition feeding of 20 to 25 seconds, while those in the present trials were allowed a 5-minute access period where multiple punctures would occur and where the possibility for acquisition of greater virus charge would exist. The results in general, that is, loss of virus being less rapid under conditions of fasting than under conditions of feeding, are comparable with those obtained by other workers (Watson, 1938, 1946; Kassanis, 1941; Kvíčala, 1948*a*, 1949*a*; MacLachlan, Larson, and Walker, 1953; Hamlyn, 1953).

Influence of Access Feeding on Virus Charge in Individual Aphids. To determine if aphids which had a 5-minute access period on a diseased plant could acquire a greater charge than those which were restricted to a 15-second acquisition feeding, the following experiment of 25 replications was done. Two aphids were selected from a noninfective colony and placed in an inverted glass vial to fast. The preliminary fasting period was 97.36 ±

28.93 minutes, for the 15-second aphids and 96.16 ± 24.55 minutes for the 5-minute access group. Following the preliminary fasting, one of the aphids was placed on an infected leaf and allowed to remain for 5 minutes, at the end of which time it was moved to a healthy mustard seedling where it

TABLE 12

DISTRIBUTION* OF INFECTIONS OF *BRASSICA NIGRA* VIRUS WHEN SINGLE GREEN PEACH APHIDS WERE FED CONSECUTIVELY ON 20 HEALTHY *B. JUNCEA* SEEDLINGS

Successive plant number																			
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Series A: one 15-second acquisition feeding																			
+	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
+	+	−	−	−	−	−	−	−	−	−	+	+	−	−	+	−	−	−	−
+	+	+	−	−	−	−	+	−	−	−	−	−	−	−	−	+	−	−	−
+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	+	−	+	+	−	−	−	+	−	−	+	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
Series B: 5-minute access period on diseased plant																			
+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
+	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	0	+	−	−	−
+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
+	−	−	−	+	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−
+	−	−	−	−	−	−	−	+	−	−	−	+	−	+	−	−	−	−	−
−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	+	−	+	−	+	−	−	−	−	−	−	−	+	−	−	−	−	+	−
−	+	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−
−	+	−	−	+	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−
−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	+	−	−	−	+	−	−	−	−	+	+	+	+	−	−	−	+	−
−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−
−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−

* The plus (+) sign indicates production of the disease, the minus (−) sign indicates no disase resulted, and the zero (0) sign indicates that the plant died. Only the positive results of 25 replications are given.

Insects were fasted and then given (A) 1 15-second acquisition feeding or (B) a 5-minute access period. The test feedings were approximately 20 seconds each.

was allowed to feed for 20 seconds before being moved to the second healthy plant. The procedure was repeated until the insect had fed on 20 healthy plants for approximately 20 seconds each. The same routine was applied to the other insect with the exception that the acquisition feeding was a watched and timed 15-second period. The total time needed to complete the series for feedings varied from 22.96 ± 3.9 minutes for the 15-second acquisition feeding insects to 24.56 ± 5.1 minutes for the 5-minute access individuals.

The order of use of the aphids was reversed with each replication. The actual test feedings, while intended for 20 seconds, varied somewhat, with a value of 17.44 ± 1.42 seconds. The time spent between punctures was 53.4 ± 12.32 seconds. It was felt that the aphids might become restless after a series of moves; consequently, a record was kept of the time required to complete the first series of 10 plants as compared with the last 10. For the aphids with the 15-second acquisition feeding, the time to complete the first 10 feedings was 10.60 ± 2.34 minutes, and 12.36 ± 2.67 minutes for the last 10. For the 5-minute access-period insects, the time to complete the first 10

TABLE 13

SUMMARIZATION* OF DISTRIBUTION OF INFECTIONS OF *BRASSICA NIGRA* VIRUS WHEN SINGLE GREEN PEACH APHIDS WERE FED CONSECUTIVELY ON 20 HEALTHY *B. JUNCEA* SEEDLINGS

Series	Successive plant number																				Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
A.....	5	4	2	3	1	2	1	1	0	2	1	1	1	1	0	1	2	0	0	0	28
B.....	7	7	3	2	6	3	4	2	1	2	0	1	3	2	2	0†	1	1	3	0	50
Total....	12	11	5	5	7	5	5	3	1	4	1	2	4	3	2	1	3	1	3	0	78

* Numbers in columns are number of plants infected out of 25.
Insects were starved, then given (A) 1 15-second acquisition feeding or (B) a 5-minute access period. Test feedings were approximately 20 seconds each.
† One test plant died.

serial feedings was 11.62 ± 3.56 minutes, and 13.16 ± 4.17 minutes to complete the last series. There was a slight tendency for the insects to take more time between feedings on the last 10 of the series of 20 plants. The temperature throughout the experiment was 23.23 ± 1.97°C.

The results (tables 12 and 13) indicated that the virus charge acquired by the insects was approximately equal as the insects which were restricted to one 15-second acquisition feeding inoculated approximately 2.8 plants per insect, and those which had a 5-minute access period on the virus source plant inoculated an average of 3.1 plants per insect. However, the range of plants infected by a single aphid was greater in the 5-minute access-time insects, being 0 to 7, while that of the 15-second acquisition-feeding insects was 0 to 5. However, in other work on serial transmission, using a single 15-second acquisition feeding, one insect was able to inoculate seven plants in a series of 20 fed upon (table 7). The results would seem to support the conclusion that multiple feedings on a diseased plant have little effect on increasing the total virus charge in any particular individual, but do serve to insure a greater number of infective insects out of any population tested. However, it is possible that factors in serial transmission experiments are too variable to demonstrate differences in virus charge, and some other test may be more definitive.

Loss of Virus During Fasting As a Measure of Virus Charge. Two of the methods used to determine the amount of virus that an infective aphid is carrying are: 1) serial transmission; and 2) post-acquisition fasting. It has been indicated in the previous section that virus retention by individual

aphids during serial transmission experiments is almost independent of the kind or number of acquisition feedings. This could be interpreted as indicating that the maximum charge attainable in the insects is limited, or it may indicate that other factors, such as loss of virus through feeding activities, are too variable to permit evaluation of original charge.

To test the second method, that of post-acquisition fasting, of determining level of charge, single aphids were given three types of acquisition feedings on a diseased *B. juncea* plant. One group was given a single 15-second acquisition feeding (14.94 ± 0.35 seconds), those in the second group were allowed five separate 15-second acquisition feedings (14.78 ± 0.87 seconds),

TABLE 14

RESULTS* OF TRIALS TO DETERMINE IF DIFFERENCES IN AMOUNT OF VIRUS ACQUIRED DURING VARIOUS TYPES OF ACQUISITION FEEDINGS CAN BE REFLECTED BY RETENTION DURING POST-ACQUISITION FEEDING FASTING

Type of acquisition feeding	Interval of post-acquisition fasting (*In minutes*)					
	0	15	30	60	120	180
1 15 second	14/30	1/30	0/30	0/30	0/30	0/30
5 separate 15 second	19/30	3/30	0/30	0/30	0/30	0/30
5-minute access	25/30	7/30	2/30	3/30	0/30	0/30

* In ratios listed, numerator is number of plants infected; denominator, number inoculated.
Green peach aphid apterae were fasted as follows: (A) 1 15-second, (B) 5 separate 15-second acquisition feedings, or (C) a 5-minute access time on a virus source, and then all lots were fasted in vials for the stated intervals prior to being transferred singly to *B. juncea* plants for approximately 1-hour test feeding.

or approximately 74 seconds of feeding, while the aphids in the third group were given a 5-minute access time on the diseased source (potential feeding time 300 seconds). The insects were fasted prior to the acquisition feedings for a period of 82.0 ± 11.35 minutes, for the 15-second group, 79.37 ± 9.95 minutes for the five separate 15-second feedings group, and 77.37 ± 12.16 minutes for the 5-minute group. Following the acquisition feeding, the insects were returned to vials and then were tested at intervals of 0, 15, 30, 60, 120, and 180 hours, by placing single insects on healthy *B. juncea* seedlings for 60.07 ±1.06 minutes. The temperature during the replications was 22.77 ± 0.85°C.

The results (table 14) show that the 5-minute access time, which increased both the number and duration of individual acquisition feedings, resulted in the greatest number of infective insects (37/180), while the five separate 15-second acquisition feedings and the single 15-second acquisition-feeding groups produced 22/180 and 15/180 infective individuals respectively.

There was some indication that the charge in the individual aphids was greater in those insects allowed 5-minute access time on the disease source since it was only with such individuals that retention of virus occurred beyond 15 minutes of post-acquisition fasting. No infections were obtained when the insects were fasted for a period of 2 or more hours. The results also might be explained on probability since the greatest number of infective individuals which could be tested occurred in the 5-minute access group.

At the 0 interval, the infections obtained in the 15-second group and those obtained in the five separate 15-second feedings were probably not due to the treatment (adjusted $\chi^2 = 1.07$, $P = 0.30$), the increased infections obtained in the 5-minute access-time group were probably due to the treatment when compared with the 15-second group (adjusted $\chi^2 = 2.130$, $P = > 0.10$).

Assuming that charge is equal in all groups, then the ratio of reduction should be the same in all groups at the end of the various fasting intervals. In the 15-second group the loss that occurred in 15 minutes was approximately 92 per cent, and the loss that occurred in the 5-minute group in 15 minutes should have been approximately 92 per cent, or approximately two individuals should have been infective at the end of 15 minutes instead of the 7. Perhaps this expected value and the actual value can be compared by the chi-square test and, if so, then the deviations to be tested would be that of 7 from 2 in a sample of 30. The adjusted χ^2 value of this comparison is 2.192, with a P value of > 0.10, indicating a slight tendency for the hypothesis of equal reduction not to be correct, or in other words that the two populations differed in virus charge. Thus there might be evidence of a more critical test for virus charge in this technique compared with that of serial transmission. One advantage that the post-acquisition fasting techniques has over serial transmission is that, relatively speaking, it should test the absolute charge of virus remaining in the insects.

This is something which the serial transmission techniques cannot do because irreplaceable virus is discharged during each successful feeding; consequently, as time goes on the charge is being dissipated at a variable rate depending on the feeding of the insects. On the other hand, the post-fasting method does not determine variations between individuals since each individual is tested for infectivity only once.

Relation of Various Factors to Aphid Transmission of Nonpersistent Viruses

Although there is no proven solution of the problem of how aphids transmit the nonpersistent viruses, there is need for a discussion of the factual and hypothetical material which has been produced by various workers, particularly during the last twenty-five years. After a résumé of experimental results on various phases of the vector-virus relationships of aphid-borne nonpersistent viruses, a modified hypothesis on the mode of transmission will be presented.

Relation of Age, Form, and Feeding of Aphid to Virus Transmission. Most aphid vector-virus work has been done with parthenogenic apterae because of their availability, in spite of the fact that alatae are held to be primary agents of field spread of nonpersistent viruses (Hansen, 1941; Broadbent, Chaudhuri and Kapica, 1950; Kennedy, 1950; Broadbent and Tinsley, 1951; Watson, Hull, Blencowe, and Hamlyn, 1951). However, there is little evidence indicating a great difference between the ability of alatae, apterae, or nymphs to transmit nonpersistent viruses (Hoggan, 1933; Severin and Freitag, 1938; Hamlyn, 1953).

Aphids lack Malpighian tubules and possess cornicles, two distinctive anatomical features. Severin and Drake (1948) failed to transmit beet mosaic virus using cornical exudate from infective aphids as inoculum. The writer is unaware of any successful attempt to transmit plant viruses through the use of honeydew, the normal excretory material of aphids, as inoculum. Severin and Tompkins (1948*a*) reported transmission of cauliflower mosaic virus with extracts from infective aphids. Other reports (Hamilton, 1935; Black, 1939) indicate that body fluids of insects extracted by pressure inhibit virus activity, but since several reports (Duggar and Armstrong, 1925; Johnson, 1938; Takahashi, 1942; Gupta and Price, 1952) indicate virus inhibitors can be extracted from various materials, the significance of virus inactivation by gross body fluids of insects is difficult to judge.

Aphid mouthparts include the stylets and the easily seen rostrum (labium). The stylets, which normally lie in the anterior groove of the labium, consist of a pair of mandibles which partially surround a pair of maxillae. Apposed maxillae form two canals, one leading to the cibarium with its pump, and the other leading to the salivary pump. Penetration usually is initiated by placing the rostrum perpendicular to the leaf surface. It is believed (Weber, 1930; Snodgrass, 1935) that penetration is accomplished by repeatedly thrusting one mandible to the depth permitted by the length of the protractor muscles, and then thrusting the other mandible. The maxillae then are thrust as a unit to the depth of the mandibles. The stylets in tissue are usually surrounded by a proteinaceous salivary sheath (Horsfall, 1923; Smith, 1933).

A rather detailed discussion of saliva flow during penetration has been given by Bradley (1952), and the writer is in agreement with most of the conclusions. In general, the observations are similar to those of Hamilton (1935), Sukhov (1944) and Storey (1939), the latter in connection with a leafhopper. Presumably, the events occurring during penetration of epidermal cells would apply to those occurring during penetration of entire plant tissue. Sukhov (1944) stated that penetration into wax was accompanied by saliva, but Bradley (1952) reported that initial penetration was made without saliva. Limited observations by the writer neither confirm nor deny the statement of Bradley, for even when aphids penetrated agar, salivary material was ejected almost simultaneously with penetration. However, when aphids attempted penetration of parlodion films, they caused indentations only, and there was no apparent evidence of saliva. Whether or not the salivary sheath completely encloses the stylets during feeding is not known, but Sukhov (1944) suggests that a continuous sheath serves as a filter and might prevent acquisition of viruses such as tobacco mosaic. However, direct proof is lacking, and there is no evidence that nonaphid transmissible viruses are not in the alimentary canals of insects feeding on diseased plants.

Bradley (1952) failed to see any movement of material to the area of the salivary sheath, although the stylets occasionally remained in place for relatively long periods of time and might be considered feeding. The writer has made similar observations.

Studies by Roberts (1940) on aphid stylets penetration into tissue indicate that the rate of penetration varies somewhat with the aphid species and possibly with the plant species. In general the rate decreased somewhat exponentially. The efficiency at reaching the phloem varied with the species, and even after 24 hours of feeding, not all tracts ended in phloem tissue (Dykstra and Whitaker, 1938; Roberts, 1940). But unless a record was kept for each penetration during the 24-hour period, the results would be subject to various interpretations. A correlation between efficiency of transmission and rate of penetration has been suggested (Dykstra and Whitaker, 1938; Roberts, 1940), but the data are too few to be conclusive.

Bradley (1952) concluded that most aphids required about 1 minute to penetrate an epidermal cell, and the work of Roberts (1940) and Sukhov (1944) indicated that about 5 minutes would be needed to go through the epidermal layer and one or two cells of the mesophyll, with a minimum of 15 minutes to attain phloem depth. Thus with nonpersistent virus epidermal cells probably can serve as a source of virus (Bradley, 1952). Application of virus directly to exposed stylets has failed to give transmission (Bradley, 1952, 1953).

The most probable area of feeding is phloem tissue (Davidson, 1923; Smith, 1926; Tate, 1927; Dykstra and Whitaker, 1938; Roberts, 1940). Aphids may penetrate inter- and/or intra-cellularly, and some species may favor one type of penetration over the other (Smith, 1926).

Part of the difficulty of observing the mechanics of aphid feeding has been the lack of clear membranes through which aphids will feed. Recent reports (Day and McKinnon, 1951; Maltais, 1952) mention plastic or rubber membranes which allow observation of feeding, and thus future work on the subject may be more feasible than it has been in the past.

The use of dyes and radioactive isotopes (Hamilton, 1930, 1935; Day and Irzykiewicz, 1953; Watson and Nixon, 1953) have given some information on aphid feeding, but correlation of these data with those of virus transmission is not on secure ground since the materials used are highly diffusable ions compared with the colloidal virus molecules.

Relation of Acquisition Threshold Period to Virus Transmission. Most, if not all, typically nonpersistent viruses can be acquired by aphids within a few seconds or minutes of feeding on a diseased plant. Although much of the available data on acquisition threshold period is in the 2- to 5-minute range (table 15), the conclusion is probably justified that the acquisition threshold period of typical nonpersistent viruses is in a 5- to 30-second range. The minimum probably is limited by the speed with which plant cuticle and the first epidermal cell wall can be penetrated. Results on three viruses (Sylvester, 1949*b*, 1950*b*; Bradley, 1952) indicated that some rise in efficiency occurred when using intervals in a 10- to 25-second rather than in a 5- to 10-second range. Penetrations maintained beyond this range were of little benefit if not detrimental. The optimum time for acquisition, while varying slightly with species, presumably results from the mechanics of feeding and the laws of probability. The decrease in efficiency noted with some species in the cases of penetration in a 30-second range (Sylvester, 1949*b*, 1950*b*) probably were due to artificially interrupted penetration

TABLE 15

ACQUISITION THRESHOLD PERIODS OF SOME NONPERSISTENT APHID-BORNE VIRUSES

Virus	Acquisition threshold period (seconds)*	Authority
Spinach blight (= cucumber mosaic)	600	McClintock and Smith, 1918
Cucumber mosaic	300	Doolittle and Walker, 1928; Hoggan, 1933
Hyoscyamus virus III	120	Watson, 1936
Red clover mosaic	300	Fukushi, 1937
Pea virus 2 (pea mosaic)	300	Osborn, 1937*a*
Cucumber mosaic (strains Y and G)	120	Watson and Roberts, 1939
Mild broad bean mosaic	600	Yu, 1939
Potato virus Y	120	Watson and Roberts, 1939; Kassanis, 1942
Onion yellow dwarf	1,800	Tate, 1940
Tobacco etch	120	Kassanis, 1941
Poison hemlock ringspot	300	Freitag and Severin, 1945*b*
Beet mosaic	120	Watson, 1946
Beet mosaic	300	Kvíčala, 1947
Lettuce mosaic	300	Kassanis, 1947
Spinach yellow dwarf	300	Severin and Little, 1947
Cabbage mosaic	300	Kvíčala, 1948*a*
Canna mosaic	300	Brierley and Smith, 1948
Cauliflower mosaic	300–600	Severin and Tompkins, 1948*a*
Cauliflower mosaic complex		
Turnip virus 1 component	300	Kvíčala, 1948*b*
Cauliflower virus 1 component	300	Kvíčala, 1948*b*
Mild stock mosaic	300	Severin and Tompkins, 1948*b*
Aspermy of tomato	180	Blencowe and Caldwell, 1949
Beet mosaic	10†	Sylvester, 1949*b*
Brussels sprouts necrosis virus	300	Kvíčala, 1949*a*
Cantaloupe mosaic	60	Dickson, *et al*, 1949
Nemesia virus (cucumber mosaic strain)	120	Watson, 1949
Papaya ringspot	120	Jensen, 1949
Subterranean clover virus	120	Watson, 1949
Brassica nigra virus	10†	Sylvester, 1950*b*
Dahlia mosaic	60	Brierley and Smith, 1950
Nasturtium mosaic	5	Jensen, 1950
Pea mosaic	120	Chauduri, 1950
Potato acuba mosaic (virus F + G)	300	Heinze, 1950
Primula mosaic	120‡	Severin and Tompkins, 1950*c*
Radish mosaic	30	Severin and Tompkins, 1950*b*
Severe stock mosaic	30	Severin and Tompkins, 1950*a*
Japanese radish stunt	300	Kasai, 1950
Cucumber mosaic	120	Bhargava, 1951
Watermelon mosaic	18–36	Anderson, 1951
Alfalfa mosaic (strain of)	15–45	Swenson, 1952
Henbane mosaic (strain of)	5–10§	Bradley, 1952
Lettuce mosaic	60–300	Fry, 1952
Western celery mosaic	10†	Simons and Sylvester, 1953
Potato virus A	15†	MacLachlan, Larson and Walker, 1953
Cabbage black ringspot	10†	Hamlyn, 1953

* Figures in column represent lowest interval tried and that interval gave positive results.
† In these cases a 5-second interval gave negative results.
‡ In this case a 30-second interval gave negative results.
§ In this case a 0–5-second interval gave negative results.

(Bradley, 1952), for during such experiments artificial interruptions of penetration occur more frequently in this range than in the 15- to 20-second range.

Relation of Inoculation Threshold Period to Virus Transmission. Inoculation threshold period studies on several viruses (Sylvester, 1949*b*, 1950*b*; Bradley, 1952; MacLachlan, Larson, and Walker, 1953; Hamlyn, 1953) indi-

TABLE 16
INOCULATION THRESHOLD PERIODS OF SOME NONPERSISTENT APHID-BORNE VIRUSES

Virus	Inoculation threshold periods (seconds)*	Authority
Spinach blight (= cucumber mosaic)	300	McClintock and Smith, 1918
Cucumber mosaic	300	Doolittle and Walker, 1928, Hoggan, 1933
Red clover mosaic	600	Fukushi, 1937
Pea virus 2 (= pea mosaic)	300	Osborn, 1937*a*
Hyoscyamus virus III	120	Watson and Roberts, 1940
Tobacco etch	120	Kassanis, 1941
Poison hemlock ringspot	300	Freitag and Severin, 1945*b*
Beet mosaic	300	Watson, 1946, Kvíčala, 1947, Severin and Drake, 1948
Spinach yellow dwarf	300	Severin and Little, 1947
Cauliflower mosaic	300	Severin and Tompkins, 1948*a*
Cauliflower mosaic complex		
Turnip virus 1 component	300	Kvíčala, 1948*b*
Cauliflower virus 1 component	300	Kvíčala, 1948*b*
Mild stock mosaic	300	Severin and Tompkins, 1948*b*
Beet mosaic	10†	Sylvester, 1949*b*
Cabbage mosaic	300	Kvíčala, 1949*b*
Papaya ringspot	300	Jensen, 1949
Brassica nigra virus	5	Sylvester, 1950*b*
Dahlia mosaic	300	Brierley and Smith, 1950*b*
Pea mosaic	120	Chaudhuri, 1950
Severe stock mosaic	600	Severin and Tompkins, 1950*a*
Japanese radish stunt	300	Kasai, 1950
Cucumber mosaic, strains of	60	Bhargava, 1951
Alfalfa mosaic, strain of	10–20	Swenson, 1952
Henbane mosaic, strain of	5	Bradley, 1952
Potato virus A	10†	MacLachlan, Larson and Walker, 1953
Cabbage black ringspot	5	Hamlyn, 1953

* Figures in column represent lowest interval tried, and that interval gave a positive result.
† In this case, a 5-second interval gave negative results.

cate that nonpersistent viruses can be inoculated within a 5- to 20-second range (table 16). Some trials suggest that a low inoculation threshold period value is somewhat easier to obtain than is a corresponding acquisition threshold period value. This may indicate that depth of penetration is not as critical in inoculation as in acquisition, or perhaps aphids secrete saliva before taking in food. Support for the latter might be deduced from experiments indicating that insects allowed normally to interrupt a penetration are more efficient in acquiring but not in inoculating virus than aphids artificially interrupted during feeding (Bradley, 1952). It has been suggested (Bradley, 1952) that if a penetration is allowed to terminate naturally, a higher proportion of aphids tested will have acquired food prior to

withdrawal than when the acquisition feeding period is artificially interrupted.

Relation of Transmission Threshold Period to Virus Transmission. In theory, the value of the transmission threshold period of a nonpersistent virus is limited only by the minimum combined value of the acquisition and inoculation threshold periods. In practice, however, it is also limited by

TABLE 17
TRANSMISSION THRESHOLD PERIODS OF SOME NONPERSISTENT APHID-BORNE VIRUSES

Virus	Transmission threshold periods (seconds)*	Authority
Spinach blight (= cucumber mosaic)	900	McClintock and Smith, 1918
Cucumber mosaic	600	Doolittle and Walker, 1928
Pea virus 2 (= pea mosaic)	300	Osborn, 1937*a*
Hyoscyamus virus III	240	Watson and Roberts, 1949
Tobacco etch	240	Kassanis, 1941
Poison hemlock ringspot	600	Freitag and Severin, 1945*b*
Beet mosaic	480	Watson, 1946
Beet mosaic	600	Kvíčala, 1947, Severin and Drake, 1948
Spinach yellow dwarf	600	Severin and Little, 1947
Cauliflower mosaic	600	Severin and Tompkins, 1948*a*
Cauliflower mosaic complex		
Turnip virus 1 component	600	Kvíčala, 1948*b*
Cauliflower virus 1 component	600	Kvíčala, 1948*b*
Mild stock mosaic	300	Severin and Tompkins, 1948*b*
Beet mosaic	42†	Sylvester, 1949*b*
Cabbage mosaic	600	Kvíčala, 1949
Papaya ringspot	420	Jensen, 1949
Brassica nigra virus	39†	Sylvester, 1950*b*
Dahlia mosaic	360	Brierley and Smith, 1950
Pea mosaic	420	Chaudhuri, 1950
Severe stock mosaic	900	Severin and Tompkins, 1950*a*
Cucumber mosaic, strains of	180	Bhargava, 1951
Alfalfa mosaic, strain of	25–65†	Swenson, 1952
Henbane mosaic, strain of	300	Bradley, 1952
Cabbage black ringspot	30†	Hamlyn, 1953

* Figure in column is lowest interval tested or published.
† In these instances specific trials were made to determine the minimum.

the rapidity with which aphids can be transferred. With those viruses which have been tested to determine a minimum, the threshold period has been reduced to a 0.5-minute range (table 17) indicating that aphids are infective immediately after acquisition of virus.

Relation of Preliminary Fasting to Virus Transmission. While early vector-virus workers (Hoggan, 1933; Watson, 1936) frequently fasted aphids to insure uniform feeding, it was Watson (1936, 1938) who first noted that preliminary fasting increased transmission efficiency if the acquisition feeding period was short. The effects of preliminary fasting have been reported for many vector-virus combinations (table 18), and it is reasonably well established that with typical nonpersistent viruses fasting before a short acquisition feeding period will increase vector efficiency.

With some viruses the rise in efficiency, as fasting time is increased, is relatively slow, reaching a maximum in a few hours. In other cases almost

TABLE 18

INFLUENCE OF PRELIMINARY FASTING ON THE EFFICIENCY OF TRANSMISSION OF SOME OF THE NONPERSISTENT VIRUSES

Vector virus	Preliminary fasting (in minutes)						Authority
	0	60	180	240	300	360	
M. persicae—Hyoscyamus III	15.7*	....	....	....	....	60	Watson, 1938
M. persicae—Hyoscamus III–V	2.1†	15.9†	....	17.6†	....	....	
	14.0	52	....	....	76	....	Watson and Roberts, 1939
Macrosiphum geisolanifolii—Hyoscyamus III–V	0.8†	7.2†	7.2†	....	....	....	Watson and Roberts, 1939
M. circumflexus—Hyoscyamus III–S	3.0†	8.0†	....	....	16.0	....	Watson and Roberts, 1939
M. persicae—Cucumber 1-G	3.5†	7.2†	....	....	11.2†	....	Watson and Roberts, 1939
M. circumflexus—Cucumber 1-G	2.8†	5.7†	....	....	9.5†	....	Watson and Roberts, 1939
M. persicae—Cucumber 1-Y	0.33†	0.33†	....	0.5†	....	....	Watson and Roberts, 1939
M. persicae—Potato-Y	4.0	34.00	....	....	62.00	....	Watson and Roberts, 1939
M. circumflexus—Potato-Y	4.0	6.0	....	....	22.0	....	Watson and Roberts, 1939
M. persicae—Tobacco etch-S	8.6	....	....	43.0	....	....	Kassanis, 1941
M. persicae—Tobacco etch-M	5.7	....	....	45.7	....	....	Kassanis, 1941
M. persicae—Potato-Y	11.8†	....	....	36.9‡	....	....	Kassanis, 1942
Aphis rhamni—Potato-Y	8.3†	....	....	34.6†	....	....	Kassanis, 1942
M. persicae—beet mosaic	6.66	50.0	....	....	....	....	Watson, 1946
M. persicae—beet mosaic	3.3	33.3	....	20.0	....	24.0	Kvíčala, 1947
Aphis rumicis—beet mosaic	0.0	2.5 (120 minutes)	....	....	....	1.7	Kvíčala, 1947
M. solani—beet mosaic	0.0	20.0	....	....	....	....	Kvíčala, 1947
M. persicae—strain of turnip virus 1	3.3	30.3	....	40.3	....	....	Kvíčala, 1948*b*
Brevicoryne brassicae—strain of turnip virus 1	0.0	13.3	....	10.0	....	....	Kvíčala, 1948*b*
M. persicae—strain of cauliflower virus 1	25.0	....	80.0	....	....	....	Kvíčala, 1948*b*
Myzus ornatus—strain of cauliflower virus 1	3.0	....	33.3	....	....	....	Kvíčala, 1948*b*
Brevicoryne brassicae—strain of cauliflower virus 1	30.0	....	55.0	....	....	....	Kvíčala, 1948*b*
M. persicae—virus necrosis of brussels sprouts	22.5	....	68(2–4 hrs.)		....	....	Kvíčala, 1949*a*
Brevicoryne brassicae—virus necrosis of brussels sprouts	47.5	....	67(2–4 hrs.)		....	....	Kvíčala, 1949*a*
M. persicae—pea mosaic	5.1†	....	....	....	45.3†	....	Chaudhuri, 1950
M. persicae—beet mosaic	10.0	66.0	80.0	70.0	70.0	87.0	Sylvester, 1949*b*
M. persicae—*Brassica nigra*	36.6	50.0	....	66.6	....	....	Sylvester, 1953*a*
Rhopalosiphum pseudobrassicae—*Brassica nigra*	6.6	20.0	....	30.0	....	....	Sylvester, 1953*a*
M. persicae—potato	0.00†	23.7†	....	14.8†	....	....	MacLachlan, Larson, and Walker, 1953
M. persicae—cabbage black ringspot	37§	46§	....	158§	....	....	Hamlyn, 1953

* Figures in columns are per cent transmission.

† More than one aphid per plant was used in test and figure listed is the calculated expectancy for a single insect.

‡ In this case the assumed transmission for calculation purposes was 99.9 per cent, actual figure listed was 100 per cent.

§ Figure represents number of local lesions produced by 30 aphids.

the full effects of preliminary fasting can be obtained within 15 minutes (table 19). The rate of increase is not constant for any particular virus or vector, but seems to be dependent somewhat on the specific vector-virus combination.

Relation of Post-acquisition Fasting to Virus Transmission. The influence of post-acquisition fasting on vector efficiency was tested before that of preliminary fasting (Doolittle and Walker, 1928). Data are available for a few vectors of such viruses as pea mosaic (Osborn, 1937*a*), red clover mosaic (Fukushi, 1937), Hyoscyamus virus III and strains (Watson and Roberts, 1939), tobacco etch (Kassanis, 1941), beet mosaic (Watson, 1946; Kvíčala, 1947; Sylvester, 1949*b*), cabbage mosaic (Kvíčala, 1948*a*), a cauliflower mosaic complex (Kvíčala, 1948*b*) (probably composed of strains of turnip virus 1 and cauliflower virus 1 groups), Japanese radish stunt (Kasai, 1950), cucumber mosaic (Bhargava, 1951), potato A (MacLachlan, Larson and Walker, 1953), and cabbage black ringspot virus (Hamlyn, 1953). The general conclusion is that vector efficiency decreases as the length of the post-acquisition fasting period increases, and loss of virus is less rapid in fasting vectors than in feeding. There is little evidence that the rate of increase in efficiency in connection with preliminary fasting is correlated with the rate of decrease in post fasting. The causes of the two phenomena may or may not be related.

TABLE 19

INFLUENCE OF SHORT PRELIMINARY FASTING PERIODS ON TRANSMISSION OF NONPERSISTENT VIRUSES

Virus	Vector	Preliminary fasting time (in minutes)						Authority
		0	5	10	15	30	60	
Beet mosaic	*M. persicae*	6.6*	53	50	....	....	47	Sylvester, 1949*b*
Beet mosaic	*M. persicae*	10.0	....	....	73	66	66	Sylvester, 1949*b*
Beet mosaic	*M. persicae*	3.3	10.0	20.0	10.0	23.0	40.0	Sylvester, 1952
Beet mosaic	*M. solani*	3.3	10.0	13.3	3.3	20.0	30	Sylvester, 1952
Beet mosaic	*M. circumflexus*	0.0	0.0	0.0	6.6	0.0	6.6	Sylvester, 1952
Beet mosaic	*Aphis apii*	3.3	3.3	3.3	0.0	6.6	0.0	Sylvester, 1952
Pea mosaic	*M. persicae*	5.1†	....	....	55.3†	....	45.3† (5 hr.)	Chaudhuri, 1950
Brassica nigra	*M. persicae*	36.6	50.0	46.5	53.3	56.3	50.0	Sylvester, 1953*a*
Brassica nigra	*Rhopalosiphum pseudobrassicae*	6.6	3.3	6.6	3.3	10.0	20.0	Sylvester, 1953*a*
Potato virus A	*M. persicae*	0.00†	0.00†	1.40†	0.70†	12.4†	23.7†	MacLachlan, Larson and Walker 1953
Cabbage black ringspot	*M. persicae*	18‡	46‡	....	108‡	145‡	144‡	Hamlyn, 1953

* Figures in columns are per cent transmission.
† These figures indicate that more than one aphid per plant was used in the test, and that figure listed is the calculated expectancy for a single insect.
‡ Figure represents number of local lesions produced by 30 aphids.

Influence of Length of Acquisition Feeding on Transmission. Many transmission trials have been made by rearing insects on infected plants, or allowing them to feed on a diseased plant for a convenient period of

time (overnight), and then moving them in lots to test plants. Much transmission work is still done in this manner, and the technique is justified by the fact that transmission usually is accomplished and a vector discovered and identified.

The technique is fairly inefficient in the use of insects, since the aphids are operating at a minimum efficiency. Maximum efficiency of aphids is gained by the use of preliminary fasting and short acquisition feeding period. However, the effect of fasting can be demonstrated only if the acquisition feeding or access time is limited to a few minutes, usually 5 or less. If the feeding is prolonged to 10 or 15 minutes, the beneficial effects of fasting are lessened, and if feeding continues for an hour or more, the beneficial effects are usually lost (Watson, 1936, 1938, 1946; Watson and Roberts, 1939; Kassanis, 1941; Kvíčala, 1947, 1948*a*, 1948*b*, 1949*a*; Chaudhuri, 1950; Kasai, 1950; Bradley, 1952; Hamlyn, 1953). The rate of efficiency loss through continuous feeding varies somewhat with specific vector-virus combinations, and more data are needed before conclusive analysis of factors involved can be made. In the work of Watson (1946) on beet mosaic there were indications that as the acquisition feeding period increased, a loss in efficiency occurred until a minimum point was reached where the vectors were transmitting with an approximate efficiency of unfasted insects. Further increase in the length of the acquisition feeding resulted in increased efficiency until finally (acquisition feeding of 24 hours) the level of efficiency was comparable with that of preliminary fasted insects given a short acquisition feeding. This phenomenon has lacked general confirmation to date, and consequently it should be interpreted with caution.

Relation of Multiple Feeding Penetrations to Virus Transmission. During a specified access time aphids may make several trial penetrations. Tests with beet mosaic virus (Sylvester, 1950*a*), a strain of henbane mosaic virus (Bradley, 1952) and the *Brassica nigra* virus in the present work, have indicated that while more infective aphids in a given population can be obtained by increasing the number of acquisition feedings, there is little increase in individual virus charge.

It may be possible that the initial puncture made after a fasting period is the critical one as far as determining infectivity, but with two viruses, beet mosaic (Sylvester, 1950*a*) and a strain of henbane mosaic (Bradley, 1952), experimental results indicated that any one of a series of punctures made on the source plant can determine infectivity.

Multiple feedings on test plants normally occur throughout most experimental work, since the test feeding period is usually an access time ranging from 1 to 24 hours, and aphids move and make several punctures during these periods. Experimental evaluations of the influence of multiple test feedings have not been extensive, and the results obtained have been somewhat conflicting. With beet mosaic virus (Sylvester, 1949*b*), increasing the number of test feedings on a test plant from one to five caused approximately a three-fold increase in positive results. However, with a strain of henbane mosaic virus (Bradley, 1952) it was reported that disturbing aphids during the test-feeding period did not result in more infections when compared with those obtained with an undisturbed group. However, the aphids were dis-

turbed at 10-minute intervals, and a 10-minute feeding was probably enough to decrease greatly the infective potential of an individual, and consequently undisturbed individuals during the period of comparison had ample time to disperse their virus charge either by long continuous punctures or several short penetrations. It seems reasonable that the number of punctures made on a test plant should affect the probability of infection. Evidence for this conclusion is also available from the results of serial transmission experiments (Sylvester, 1950*a*).

In the relatively limited work that has been done on the effect of duration of a single test-feeding puncture on transmission efficiency the percentage of transmission obtained with intervals of 10 seconds or lower was less than that obtained with longer feeding periods (Sylvester, 1949*b*, 1950*b*). This may be due to the probabilities associated with the time required for the insects to overcome the physical barrier to penetration.

Serial Transmission of Viruses. In early experimental work, the failure to obtain serial transmission of nonpersistent aphid-borne viruses was one of the main reasons for believing that transmission was due to contamination of stylets. Hoggan (1933) failed to get serial transmission of cucumber mosaic virus when infective insects were moved from plant to plant at 2-hour intervals.

Watson (1938) serially transmitted Hyoscyamus virus with aphids, and reported that the time spent on the first plant affected the amount of transmission to the second. Since this early work, serial transmission has been demonstrated for a number of nonpersistent viruses (Watson, 1936; Watson and Roberts, 1940; Fukushi, 1937; Osborn, 1937*a*, 1937*b*; Severin and Freitag, 1938; Kassanis, 1941; Freitag and Severin, 1945*b*; Severin and Little, 1947; Severin and Tompkins, 1948*a*, 1948*b*, 1950*a*, 1950*b*; Kvíčala, 1947, 1948*b*, 1949*b*; Brierley and Smith, 1950; Sylvester, 1950*a*; Bradley, 1952, 1953; Hamlyn, 1953; MacLachlan, Larson, and Walker, 1953).

Some workers (Watson, 1938; Watson and Roberts, 1939, 1940) have used the first healthy plant fed on to reduce the charge of virus. Other workers moved infective aphids at given intervals to a series of five or more plants. Usually these intervals have been in a 5- to 10-minute range, but occasionally hourly intervals have been used. The collective evidence indicates that while nonpersistent viruses can be serially transmitted, the longer the test-feeding intervals, the less likely is serial transmission to occur. Tests with beet mosaic virus (Sylvester, 1950*a*), a strain of henbane mosaic virus (Bradley, 1952), cabbage black ringspot virus (Hamlyn, 1953), and the *Brassica nigra* virus in the present work, to determine how many plants can be successively infected by a single aphid have indicated that the number of plants infected per individual varies greatly, and thus it would seem that individual aphids differ considerably in amount of virus carried. On a total sample basis, the first few plants fed on are the most likely to be infected, but there is no method of predicting individual patterns of dispersal.

Relation of Virus Charge to Transmission. It has been assumed by most workers that variations exist in the ability of individual insects to cause infection, and many results in serial transmission experiments indicate this to be true. The methods of increasing virus charge are concerned with

variations in the type of acquisition feeding and treatment of the insects before acquisition feeding. Watson (1938) found that individual aphids varied somewhat in their capacity to transmit Hyoscyamus virus III, and that if an aphid, fasted or nonfasted, once produced an infection, the probabilities were slightly higher that it would do so again. When individuals were used in a second trial there was a tendency for the infectivity level to drop. This was interpreted as possible satiation of appetite, and might indicate that variations of virus charge exist within as well as between individuals. Bradley (1952), using a strain of henbane mosaic virus, reported that nonfasted individuals could obtain as much charge per acquisition feeding as fasted individuals. This might indicate that infective nonfasted insects are in the same physical state as infective fasted aphids. However, other work (Watson, 1938), using post-acquisition fasting as a test of infectivity, indicated that the rate of loss of virus charge was somewhat dependent on the condition of the insect prior to acquisition, the loss being more rapid in the case of nonfasted insects.

Previous results (Sylvester, 1950*a*) and those in the present work indicate that varying the number and kind of acquisition feeding does not materially increase the virus charge per aphid, if serial transmission is used as the method to determine level of charge. But if post-acquisition fasting is used, the results in the present work might be interpreted as indicating that increasing the number, or changing the type of acquisition feeding, not only increases the probability of obtaining infective individuals, but increases the charge within a given aphid.

Vector Efficiency and Vector Specificity. Vector efficiency refers to the probability of obtaining infection with a given virus using a given vector under a given set of conditions. Numerous factors influence transmission, and consequently vector efficiency is subject to wide variation. Comparative studies indicate that among aphid species differences in ability to transmit viruses exist (Watson and Roberts, 1939; Doncaster and Kassanis, 1946; Kvíčala, 1945, 1947, 1948*b;* Sylvester and Simons, 1951; Sylvester, 1952; Simons and Sylvester, 1953). Other, less comparative, data on vector efficiency can be found in the works of Severin and Freitag (1938), Kassanis (1941), Freitag and Severin (1945), Severin and Little (1947), Severin and Drake (1948), Severin and Tompkins (1948*a*, 1948*b*, 1950*a*, 1950*b*, 1950*c*), Heinze (1950), Stoner (1951), and Swenson (1952).

Watson (1938) reported that the efficiency of transmission of the Hyoscyamus virus III varied with virus strains, and interpreted the results as indicating that the two strains occurred in different concentrations in the common host. However, Kassanis (1941) presented data indicating that two strains of tobacco etch virus, which occurred in unequal concentrations in tobacco, were transmitted with equal efficiency.

In connection with vector specificity, Doncaster and Kassanis (1946) compared *Myzus ascalonicas* Doncaster and *Myzus persicae,* and found that the former would transmit dandelion yellow mosaic virus, while the latter would not; that both species could transmit cucumber virus I and Hysocyamus virus III, with varying degrees of relative efficiency; and that *M. persicae* would transmit potato virus Y, severe etch virus of tobacco, lettuce mosaic

virus, and sugar beet mosaic virus, while *M. ascalonicus* would not. Kvíčala (1945, 1948*b*) reported in connection with transmission of a virus complex composed of turnip virus 1 and cauliflower virus 1 strains that *M. ornatus* Laing was a selective vector, transmitting only the cauliflower virus 1 component. It was also found that *M. ornatus* readily inoculated Chinese cabbage but not cauliflower. Kvíčala suggested that this might be due to inadequate feeding on waxy leaves of cauliflower, or that cauliflower was less susceptible.

TABLE 20

MAXIMUM REPORTED RETENTION OF SOME OF THE NONPERSISTENT VIRUSES BY FEEDING APHIDS

Virus	Vector	Retention	Authority
Hyoscyamus virus III	*Myzus persicae*	6 but not 12 hours	Watson, 1936
Red clover mosaic	*Myzus persicae*	30 minutes	Fukushi, 1937
Pea virus 2 (pea mosaic)	*Macrosiphum pisi*	25 minutes	Osborn, 1937*a*
Vein mosaic of red clover	*M. pisi*	more than 1, but less than 24 hours	Osborn, 1937*b*
Western celery mosaic	*Aphis ferruginea striata*	up to 10 hours	Severin and Freitag, 1938
Tobacco etch	*Myzus persicae*	15 but not 30 minutes	Kassanis, 1941
Poison hemlock ringspot	*Rhopalosiphum conii*	8 hours	Freitag and Severin, 1945*b*
Beet mosaic	*Myzus persicae*	3 hours	Kvíčala, 1947
Spinach yellow dwarf	*M. persicae*	2 hours	Severin and Little, 1947
Cauliflower mosaic	*Brevicoryne brassicae*	2 but not 3 hours	Severin and Tompkins, 1948*a*
Mild stock mosaic	*Rhopalosiphum pseudo-brassicae*	10 minutes	Severin and Tompkins, 1948*b*
Cabbage mosaic	*Myzus persicae*	70 minutes (10-minute transfers)	Kvíčala, 1949*b*
Papaya ringspot	*M. persicae*	5 minutes	Jensen, 1949
Dahlia mosaic	*M. persicae*	more than 2 but less than 3 hours	Brierley and Smith, 1950
Radish mosaic	*Brevicoryne brassicae* and *Rhopalosiphum pseudo-brassicae*	3 hours	Severin and Tompkins, 1950*b*
Severe stock mosaic	*Myzus persicae*	2 hours	Severin and Tompkins, 1950*a*
Japanese radish stunt	*Myzus persicae*	1 hour	Kasai, 1950
Potato A	*Myzus persicae*	20 minutes	MacLachlan, Larson, and Walker, 1953
Cabbage black ringspot	*Myzus persicae*	55–110 minutes	Hamlyn, 1953

Sylvester and Simons (1951) found *Rhopalosiphum pseudobrassicae* (Davis) in general to be a less efficient vector of *Brassica nigra* virus than *M. persicae,* but that the comparative efficiency was somewhat dependent on the host inoculated. One of the most quoted examples of lack of vector specificity in nonpersistent viruses is that of onion yellow dwarf virus where some 48 of 50 species tested were reported to be vectors (Tate, 1940).

Some of the more recent trials which have compared, in factorially designed experiments, several vector preliminary fasting-acquisition feeding combinations are of special interest, and have indicated that the rate of gain and loss of efficiency vary with the aphid species. With the assumption that gain and loss in efficiency found with preliminary fasting and subsequent lengthening of the acquisition feeding are due to effects on virus inactivators, some workers (Watson and Roberts, 1939; Chaudhuri, 1950) have presented ratio values, presumably to be used to compare rates of inactivation among

various vectors species. While it would appear that the experimental results are too variable to be of value in forming any generalizations as to specific rates of inactivation, Smith and Lea (1946) attempted mathematically to evaluate a rate of inactivation using a selected group of data. Although trends exist which indicate gain and loss of efficiency to be somewhat exponential, it would seem that attempts to calculate specific values for these rates would be subject to error of such magnitude as to make it valueless except from the standpoint of methodological approach.

Virus Retention by Aphid Vectors. Virus retention has been mentioned in connection with acquisition feeding, effect of preliminary fasting, post-acquisition fasting, and serial transmission, and table 20 lists results of feeding-retention experiments on several nonpersistent viruses.

Several viruses are reported as not persisting in their aphid vectors, viruses such as dandelion yellow mosaic (Kassanis, 1947) and some of the strawberry viruses (Prentice and Harris, 1946), but there is still some uncertainty as to whether these are persistent or nonpersistent viruses since there is a positive correlation between length of acquisition feeding and transmission efficiency. The placing of aphid-borne viruses into two groups, that is, persistent and nonpersistent (Watson and Roberts, 1939, 1940) may be premature; instead there may be several groups of aphid-borne plant viruses.

Although testing for virus retention during a period of fasting is a relatively old technique (Doolittle and Walker, 1928; Hoggan, 1933), data on the subject are not extensive. The several references which are available (Fukushi, 1937; Watson, 1938, 1946; Watson and Roberts, 1939; Kassanis, 1941; Kvíčala, 1947, 1948*b*, 1949*b*; Kasai, 1950; MacLachlan, Larson, and Walker, 1953; Hamlyn, 1953) indicate that aphids retain nonpersistent viruses for longer periods if fasted rather than fed.

Other Factors in Transmission: Relation of Numbers of Insects to Virus Transmission. Under conditions of low probability of transmission, it is common practice to use groups of insects. The influence of increasing the number of aphids on transmission of nonpersistent plant viruses has been investigated comparatively with such viruses as: cucumber mosaic (Hoggan, 1933), Hyoscyamus virus III (Watson, 1936), cucumber virus 1 G (Watson and Roberts, 1939), beet mosaic (Watson, 1946; Kvíčala, 1947), cauliflower mosaic (Kvíčala, 1948*b*), cabbage black ringspot virus (Hamlyn, 1953), as well as some others. Single aphids as well as groups were used in the transmission of western celery mosaic virus (Severin and Freitag, 1938), crinkle-leaf strain of western celery mosaic virus (Freitag and Severin, 1945*a*), poison hemlock ringspot virus (Freitag and Severin, 1945*b*), cauliflower mosaic virus (Severin and Tompkins, 1948*a*), radish mosaic virus (Severin and Tompkins, 1950*b*), mild and severe stock mosaic viruses (Severin and Tompkins, 1948*b*, 1950*a*), beet mosaic virus (Severin and Drake, 1948), and spinach yellow dwarf virus (Severin and Little, 1947). These tests, not comparatively designed, can not be interpreted as strictly as those designed specifically to determine the influence of numbers on transmission.

Watson (1936) presented an adaptation of the binomial theorem to determine the transmission expectancy of any group of individuals, and it has been generally conceded that variations of the actual results from the pre-

dicted are not of sufficient magnitude to negate the assumption that individuals in a group cause separate and independent infections. Apparently there is no accumulation of small individual dosages, the sum of which would cause infection whereas each alone would not (mass action). This has been held to be generally true for most vector-virus combinations, including the nonpersistent aphid-borne viruses, although some modification may be necessary (Kirkpatrick and Ross, 1952). If P is the probability of obtaining an infection with one individual (per cent transmission divided by 100), and q is the probability of not obtaining an infection with one individual, then $P = 1 - q$. With x individuals, the probability of obtaining an infection with a qroup p, is $1 - q^x$. The experimental value of P differs greatly from time to time and sample to sample, and transmission expectancy predictions are applicable only to limited experimental conditions which often are difficult to duplicate.

Variations in Transmission Due to Temperature and Light. Watson (1936) obtained negative correlations with light and temperature in the transmission of Hyoscyamus virus III, and attributed the results primarily to effects on plants, since increasing the amount of light on aphid colonies during winter did not lower transmission. The specific effects of light and temperature on transmission have not been critically investigated. The general opinion is that winter months are best for working with mosaic-type nonpersistent viruses.

Temperature has been used to a limited extent in trials on the effects of fasting. In general, the rate of increase in efficiency due to preliminary fasting is positively correlated with temperature, but the temperature ranges tested have not been wide nor subjected to critical control. Likewise, it has been found that the rate of loss of infectivity during post-acquisition fasting is positively correlated with temperature, but again the data are few and the tested ranges of temperature limited (Kassanis, 1941).

Effects of Humidity on Transmission. Little has been done with the effects of humidity on virus transmission by aphids. Watson (1936) reported that the time required by aphids to settle down to a more or less permanent feeding site (variously called penetration or prepenetration time, with a value of approximately 5 minutes for *M. persicae* (Watson, 1936; Severin and Drake, 1948; Sylvester, 1949*a*), was negatively correlated with relative humidity. Other work, but not specifically in connection with virus transmission on aphids and relative humidity, includes those of Davies (1935) and Broadbent (1949).

Effect of Plants on Virus Transmission: Virus Source Plants. The effects of virus source plants on transmission are not well known. Watson (1936) in Hyoscyamus virus III transmission ran local lesion assays to determine the best leaf age to use as virus source for aphid feeding. In experimental results, large differences between replications may be partially explained on the basis of differences in virus sources. Recent work on the *Brassica nigra* virus (Sylvester, 1953*a*) indicated that differences existed among virus source plants which had been simultaneously raised and inoculated and which were selected because of uniformity in time and type of symptom development.

The species of plant used for a virus source might be expected to influence

transmission. Watson (1936) found no difference between tobacco or Hyoscyamus as a virus source. Kvíčala (1948*b*) reported cauliflower and Chinese cabbage to be comparable sources of a strain of turnip virus 1, and it was found (Sylvester and Simons, 1951) that pak choi and *Brassica juncea* Coss. were approximately equal as sources for the *B. nigra* virus. On the other hand, Freitag and Severin (1945*b*) found parsley to be a poor source of the poison hemlock ringspot virus when compared with poison hemlock and celery.

Effect of Test Plants on Virus Transmission. Species of plants differ in their susceptibility to infection by aphid transmitted viruses. The data of Hoggan (1933) indicate that cucumber was somewhat more susceptible to infection with cucumber mosaic virus than tobacco. Kvíčala (1948*b*) reported that cauliflower was less susceptible than Chinese cabbage to infection with a strain of cauliflower virus 1 when inoculated by *Myzus ornatus*. *Brassica nigra* virus can be inoculated more readily by the green peach aphid into smooth leaf mustard than into pak choi (Sylvester and Simons, 1951). In other cases susceptibility to insect inoculation may be equal. Severin and Tompkins (1948*a*) reported that cauliflower and stock were approximately equal in susceptibility to cauliflower mosaic virus when inoculated either by *Brevicoryne brassicae* or *Rhopalosiphum pseudobrassicae,* but a differential existed in favor of cauliflower when using *Myzus persicae*. Data on the *Brassica nigra* virus (Sylvester, 1953*b*) indicated that not only do various species and/or varieties of *Brassica* differ in susceptibility to insect inoculation, but also that one variety of plant was aphid but not juice inoculable. In general, juice inoculation has been more certain to induce infection than insect inoculation (Severin and Freitag, 1938; Freitag and Severin, 1945*a*, 1945*b*; Severin and Little, 1947; Severin and Tompkins, 1948*a*, 1948*b*, 1950*b*; Severin and Drake, 1948). Such comparisons between insect and juice inoculation probably are only of empirical value since the total amount of virus available for inoculation and the surface exposed to infective virus in the one method is not comparable with that in the other.

When using one test plant species, variation within plants and between plants probably exists, but little evidence of such is available to date. Watson (1936) using Hyoscyamus virus III concluded that variations between plants were no greater than within plants. The results were not obtained with aphids, but with juice inoculation, and whether or not results obtained with one method of inoculation are applicable to another is not known. In the case of one phenomenon, the effect of light on susceptibility of host plants and the results obtained using juice inoculation have not been comparable with those obtained with insects. With juice inoculation it has been possible to show a correlation of light intensity with the number of local lesions obtained (Samuel, Best, and Bald, 1935; Bawden and Roberts, 1947; Sill and Walker, 1952). However, to date attempts to demonstrate light sensitive changes in susceptibility of plants to aphid inoculation have failed (Bradley, 1952; Sylvester, 1953*a*). Possibly the age of the test plant influences transmission, but there are few data available on the subject. In work with the *Brassica nigra* virus (Sylvester, 1953*a*) test plants one to six weeks old were approximately equal in susceptibility to insect inoculation. However, Carter (1937)

stated that with *Commelina nudiflora* mosaic, transmission efficiency decreases as pineapple seedlings grew older.

Hypotheses on the Mode of Transmission of Nonpersistent Viruses. Doolittle and Walker (1928) and Hoggan (1933), working with aphid transmission of cucumber mosaic virus, concluded that the aphids carried the virus on the mouthparts and transmission was mechanical. This conclusion was reached because: 1) the aphids could acquire and transmit virus within 10 to 30 minutes; and 2) the insects lost infectivity after one test feeding or after 6 to 18 hours of fasting. However, Hoggan (1933) recognized that the concept of mechanical transmission did not explain vector specificity or the failure of aphids to transmit readily such viruses as tobacco mosaic. The reasons offered as possible explanation were that the virus was not in tissues fed upon, or that the virus was inactivated through a complex interaction among the virus, aphid, and plant sap. Furthermore, it was stated that lack of serial transmission could be explained by assuming that virus was inactivated through prolonged contact with the insects, and that the rate of inactivation was less in fasting than in feeding aphids.

Most workers subsequently favored the simpler of the two explanations offered by Hoggan (1933), until the works of Watson (1936, 1938, 1946) and Watson and Roberts (1939, 1940).

In 1936 Watson (1936), using Hyoscyamus virus III and fasted aphids, concluded that mechanical transmission was improbable because: 1) aphids could inoculate more than one plant in a series; 2) acquisition feeding periods beyond 2 to 5 minutes decreased infectivity of fasted aphids; and 3) increasing the test feeding period on the first healthy plant increased the probability of obtaining infection on that plant, but decreased the probability of infecting a second test plant. Watson concluded that fasting aphids increased transmission efficiency, and lengthening the acquisition feeding period decreased infectivity because either: 1) virus concentration was highest in tissues reached after 2 to 5 minutes of feeding; or 2) contact with the insect adversely affected the virus (Hoggan, 1933). Assuming the insects affected the virus, it was further suggested (Watson, 1936) that inactivation could be by: 1) fasting labile digestive enzymes; or 2) antibodies formed at a rate less than that of virus uptake. Neither was held probable because the transmission threshold period was believed too short for virus to pass from the stomach into the blood and then to the salivary glands from where it was ejected.

The probability of transmission increasing with the length of the first test feeding might be due to (Watson, 1936): 1) breaking of an incomplete antibody-antigen complex by healthy plant juice (rejected if the low value for the transmission threshold precluded the probability of virus being in the blood); or 2) an increase in plant tissue susceptibility through continuous injury during long feeding periods.

After additional experiments Watson (1938) proposed that in aphids some substance (enzyme ?) was present which caused inactivation. Other work with plant virus had demonstrated that certain substances, including insect juices (Hamilton = Watson, 1935) and some enzymes such as trypsin (Lojkin and Vinson, 1931; Stanley, 1934) caused reduction in activity of specific

virus dilutions, the amount being dependent on the initial concentration of the material, and inactivation was immediate and not furthered by incubation. This type of inactivation was assumed to be analogous to that occurring with aphid transmission of Hyoscyamus virus III. Most of the facts concerning the effects of preliminary fasting and increased acquisition feeding on virus transmission thus could be accounted for, since fasted insects, having less inactivator would be better vectors, but efficiency would decrease during feeding because feeding would stimulate production of the inactivator.

The exact nature of the enzyme was not known (trypsin was suggested as a possibility), and the problem still persisted as to how the inactivator came into contact with the virus. Three possibilities were suggested: 1) salivary secretions (rejected because of the reported absence of proteinases in those of phytophagus insects); 2) regurgitated stomach material (regurgitation was considered earlier by Severin (1931) to account for some anomalies in curly top virus transmission), but this was rejected because the action of the alimentary canal in aphids was such as to move material predominately one way; or 3) the blood (rejected because of the low transmission threshold period value).

Further work by Watson and Roberts (1939) compared transmission of three viruses (Hyoscyamus virus III, potato virus Y, and cucumber virus 1) by three vectors (*Myzus persicae, M. circumflexus,* and *Macrosiphum gei*) in response to preliminary fasting and length of acquisition. The terms *persistent,* that is, viruses retained for relatively long periods of time in vectors, and *nonpersistent,* that is, viruses rapidly lost by vectors, were proposed and subsequently adopted by most vector-plant virus workers, but perhaps with a too generalized application. The inactivator was assumed, and the discussion was concerned with an explanation of the different degrees of transmission efficiency and response to preliminary fasting in the various vector-virus combinations. The following postulates were made: 1) the inactivator is produced during feeding, but not, or at a lower rate, during fasting; 2) the quantity and rate of production of inactivator varies with the insect species; and 3) the inactivator is common to all aphids, and acts on all viruses in a similar manner (differences exist in quantity but not in quality). With these assumptions as a basis, vector efficiency depends on: 1) the quantity of virus available to the insects; 2) the rate at which the inactivator is produced during feeding; and 3) the rate of loss of inactivator during fasting.

However, the authors (Watson and Roberts, 1939) pointed out certain inconsistencies in the data. One species (*Myzus circumflexus*) was equal to another (*M. persicae*) when transmitting one virus (cucumber virus 1 G), but was less efficient when transmitting the other two. In explanation it was suggested that the viruses were located in different depths of tissue, and aphids penetrated at different rates. Since the amount of inactivator present increased with feeding, the aphid reaching the area of optimum virus concentration first would be the better vector, and a differential could be demonstrable if the viruses were in deep tissues. While logical, the assumption is not entirely warranted for general application, for it has been shown using the same two vectors, and two other viruses (beet mosaic and western celery

mosaic), similar prefasting times, and 10- to 30-second acquisition-feeding periods, that reversal in relative efficiency occurs, and in this case depth of tissue penetration could have little effect since the insects presumably are feeding in epidermal cells (Sylvester, 1952; Simons and Sylvester, 1953).

Another anomaly evident was that one species (*Macrosiphum gei*) transmitted two viruses (Hyoscyamus virus III and potato virus Y) with equal efficiency in spite of the fact that one virus occurred in much lower concentrations in the plant than the other. The explanation offered for this (Watson and Roberts, 1939) was that one virus might be more readily available to this particular species than the other (but it was proposed that aphids fed in essentially the same manner), or that one virus was less readily affected by the inactivators. This latter necessitates a qualitative as well as a quantitative difference in the behavior of the inactivator of different species on different viruses, a complication which generally has been avoided by workers (Watson and Roberts, 1939; Watson, 1946; Smith and Lea, 1946).

The problem remained as to how the inactivator came into contact with the virus. Internal contact was not held probable, and the other possibility considered was that the substance was ejected with the saliva and that the virus was inactivated in the source plant. This was a distinct possibility, but necessitated the assumption that mechanical transmission was the actual mechanism of virus transfer. Although Watson and Roberts (1939) now recognized the possibility of simple serial transmission occurring under conditions of mechanical transmission, additional evidence obtained in serial transmission trials indicated that loss of virus per feeding was less if insects were rapidly moved than if allowed to feed more or less continuously. This was considered strong evidence against the idea of simple mechanical transmission since the rapidly moved insects fed on more plants and presumably had more chance to clean their stylets of contaminating virus. However, in this work the authors failed to consider the amount of stylet activity that could occur during long penetrations, and it is possible in the light of recent observations (Bradley, 1952) that the total amount of decontaminating surface presented to the stylets during one deep penetration is equivalent to, or more than, that which might be presented during a rapid series of limited penetrations.

In connection with the interpretation of the data on the basis of inactivation, Watson and Roberts (1940) suggested that a 2-minute feeding period was not sufficient to stimulate production of the inactivator and, consequently, it must be concluded that the loss of infectivity by the group of insects moved at 2-minute intervals was due to exhaustion of virus supply by mechanical dispersion, ejection, or ingestion. Watson and Roberts (1940) clarified and simplified their position and stressed the division of insect-borne plant viruses into two groups, persistent and nonpersistent. They suggested that the concept of mechanical transmission rested on three postulates: 1) aphid transmission of the viruses occurred in periods of time too brief to allow the virus to go through the insects; 2) failure to obtain serial transmission (earlier workers used long test feeding periods); and 3) retention during fasting was longer than during feeding, indicating that the stylets

were not being decontaminated as rapidly during fasting, but that the virus was being lost at rates comparable with those of inactivation *in vitro*.

Watson and Roberts (1940) dismissed the first postulate on the grounds that neither the rate nor the path of virus movement through insects was known. However, in other work (Watson, 1936, 1938; Watson and Roberts, 1939) they accepted the validity of this assumption for use in interpretation of the inactivator hypothesis, so apparently a low transmission threshold period value can be used in support of, but not to the exclusion of, either hypothesis. The second postulate (lack of serial transmission) was rejected because serial transmission had been demonstrated, but, as had been pointed out (Watson and Roberts, 1939), serial transmission was probable under certain conditions even if the process were mechanical. The third argument (retention during fasting longer than during feeding and for a period comparable with that of survival of virus *in vitro*) was rejected because the virus was shown to have been lost by fasting insects in less time than that required for inactivation *in vitro*. They referred to Doolittle and Walker (1928), but it is obvious that these workers were in error in the figure of 6 to 8 hours for the time of inactivation of cucumber mosaic virus *in vitro*. Considering the amount of virus in or on the stylets of an insect, and the variability of biological tests, it is remarkable that fasting aphids retain virus for as long as they do, even if the loss is only due to *in vitro* inactivation and not complicated by an inactivator.

Thus it would appear that these facts used to reject a mechanical transmission hypothesis and to support one of inactivation by insects could be used to support either hypothesis with somewhat comparable facility and logic.

The inactivator hypothesis was extended (Watson and Roberts, 1940) to include the transmission of persistent as well as nonpersistent viruses, the difference between the two being one of rate of inactivation. This was supported by Smith and Lea (1946) who extended the argument to indicate that nonpersistent viruses were more rapidly inactivated by aphids and occurred in higher concentrations in plants and therefore were juice inoculable, while the persistent viruses were more slowly inactivated by aphids, occurred in lower concentrations in plants, and consequently had a lower probability of being juice transmissible. The fact that some of the persistent aphid-borne viruses are juice inoculable (Osborn, 1938; Kassanis, 1949) under certain conditions but with varying results would tend to support this hypothesis.

In 1946 Watson after experiments with a persistent (beet yellows) and nonpersistent (beet mosaic) virus with a common vector (*M. persicae*), and a common host (sugar beet) concluded, with the assumption of the inactivator hypothesis, that the terms persistent and nonpersistent did not express the true difference between the two groups of viruses. Watson found that retention was relative to the extent that there was a possible overlapping in the time of vector retention among persistent and nonpersistent viruses, and therefore suggested that the effect of preliminary fasting on efficiency would be a better basis for distinguishing the two groups. It has been pointed out (Sylvester, 1949*a*) that the original implication carried by the terms is still generally valid if strict limits as to the exact time are not set. However, further qualification as to a positive or negative response to preliminary

fasting may improve the definition of the two groups. Watson (1946) also noted that it was doubtful whether or not the differences found to date between the persistent and nonpersistent viruses would serve as a satisfactory basis for the classification of insect-borne plant viruses, but there were certain practical applications.

Other workers (Kassanis, 1941; Kvíčala, 1947, 1948*a*, 1948*b*, 1949*a*, 1949*b*; Chaudhuri, 1950; Bhargava, 1951; Hamlyn, 1953) have given additional data and in the main the results have been interpreted using the inactivator hypothesis. Kassanis (1947) noted an anomaly in dandelion yellow mosaic, since preliminary fasting failed to improve transmission, but the insects retained the virus for only an hour, and the length of the acquisition feeding was positively correlated with efficiency. Consequently, on the basis of retention it was nonpersistent, but on the basis of response to preliminary fasting and length of the acquisition feeding, it was persistent. Similar situations have been reported with some of the strawberry viruses (Prentice and Harris, 1946).

In summary, the inactivator hypothesis can be used to explain many facts concerning transmission on nonpersistent viruses by aphids, but the two problems which are the least satisfactorily explained by the concept of a generalized inactivator in aphids which acts on all plant viruses in a similar manner are: 1) vector specificity, ranging from degree of efficiency to inability of some species to transmit some viruses; and 2) the lack of aphid transmission of such viruses as tobacco mosaic, potato-X, turnip yellow mosaic, southern bean mosaic, tobacco necrosis, tomato bushy stunt; viruses which occur in relatively high concentrations in the hosts, are readily juice transmissible, and are quite stable *in vitro*.

Bradley (1952), working on a strain of henbane mosaic virus (Hyoscyamus virus III strain), recently reviewed briefly the mechanical and inactivator hypotheses and, using his data and observations, concluded that transmission on nonpersistent viruses was largely mechanical, with the virus being carried within the stylet canals (Hoggan, 1933). The following explanation of transmission was proposed: 1) the salivary sheath, which acts as a filter (Sukhov, 1944) is absent during brief penetrations, and thus the stylet canal, or canals, may become obstructed, and require the aphid to force material out; 2) as long as feeding occurs without salivary sheath, the insect is likely to acquire and transmit virus; 3) when feeding is normal the sheath prevents clogging and the probability of transmission is lowered; and 4) fasting increased the probability of penetration being made without sheath material, and short penetrations are more likely to be unaccompanied by saliva than are long ones.

With these assumptions Bradley (1952) was of the opinion that the effects of preliminary fasting and long acquisition feeding could be accounted for and consequently mechanical transmission was a possibility. The failure of the hypothesis to explain either vector specificity or the lack of transmission of some readily juice transmissible viruses was admitted by Bradley (1952) with the stipulation that with the data available to date, it would be premature to develop a satisfactory explanation.

Watson and Nixon (1953), in connection with a paper on aphid feeding experiments with ^{32}P, briefly mentioned the work of Bradley (1952), but concluded, in the light of present knowledge, that the inactivator hypothesis put forth by Watson and Roberts (1939) still seemed the most plausible and the least complicated. Parenthetically Watson and Nixon (1953) introduced a modification of the hypothesis as originally stated (Watson and Roberts, 1939) to the extent that the virus presumably could be inactivated in the test plant (Smith, 1933; Sylvester and Simons, 1951; Simons and Sylvester, 1953).

It is apparent that none of the explanations put forth fully and satisfactorily explains the facts regarding transmission of the nonpersistent aphid-borne viruses as they are now known, and the most perplexing problems have been those of vector specificity and lack of transmission of certain highly infectious viruses. The answer may be in a combination of the mechanical and inactivator hypotheses. For purposes of discussion the following assumptions are made: 1) transmission is mechanical in the sense that virus is carried within the food canal of aphids (Hoggan, 1933; Bradley, 1952); 2) aphids feed in a similar manner and in similar areas during initial stages of penetration, although they may exhibit variations in rate of penetration, efficiency in attaining phloem, and in the exact path of penetration (inter-, intra-cellular, or a combination of the two); 3) aphids acquire a similar charge of virus when feeding but a short time on a given virus source plant (Sylvester, 1950*a;* Bradley, 1952), that is, between species variations in charge are probably no greater than within species variations, and the amount of virus acquired is independent of the kind of virus with the restriction that it must be present in similar amounts in cells penetrated; and 4) the action of inactivators which are present in the salivary secretions (Hoggan, 1933; Watson, 1938) is not upon the virus, but rather upon the host plant cells into which the virus is injected (Smith, 1933; Sylvester and Simons, 1952; Simons and Sylvester, 1953), namely, the insect renders the host plant cell resistant or practically immune to infection.

Admittedly the evidence in support of this "incompatibility hypothesis" is meager, but the implications would be such as to indicate that all viruses such as tobacco mosaic, potato-X, et cetera are taken up by aphids when feeding, but cannot be demonstrated as being in the insects by transmission tests because the combination of salivary secretions and the contents of inoculated plant cell, or cells, is incompatible with the virus to such an extent that transmission is highly improbable. In accordance with this view it should be possible to demonstrate transmission of such a virus as tobacco mosaic with the qualification that the plant inoculated must be of such a nature that the virus-saliva-host plant combination is not incompatible. Perhaps evidence for this possibility can be deduced from the early work of Hoggan (1933) reporting that transmission of tobacco mosaic virus to tomato was possible in a limited number of cases when using large aphid populations, but not to tobacco. This might have been due to accidental contamination or due to the possibility that cucumber mosaic virus was present as well as tobacco mosaic virus, but with the incompatibility hypothesis the results could be expected. Evidence that virus acquisition is somewhat independent of the plant species

might be deduced from the results of Bennett and Wallace (1938) who demonstrated that the green peach aphid, as well as other insects, could acquire curly top virus but could not transmit it. It also might be deduced from recent work of Marmarosch (1952) where certain viruses could be inoculated into nonvector species and would stay infectious within the body of the species for a relatively long period of time, but the insects could not serve as vectors.

Other evidence for the hypothesis might be taken from results of recent efforts to determine the mode of action of various inactivators of such viruses as tobacco mosaic virus, for which case it has been concluded that the most likely causes of inactivation are due to effects of inactivators on the host plants rather than on the viruses (Slagle, Wolcyrz and Price, 1952). It would seem probable that the presence of such inactivators in virus source plants would have little effect on the recovery of active virus from the plants since separation of virus and inactivators can be made by dilution. Still other evidence might be deduced from the recent work of Kirkpatrick and Ross (1952) on potato leaf roll virus transmission which indicated that the presence of a large number of insects (infective or noninfective) on a test plant decreased the probability of obtaining an infection, suggesting that the test plant was being modified.

Perhaps additional direct evidence of the independence of plant species and virus acquisition from a source is that indicated in work with the *Brassica nigra* virus, where two species of plants served equally well as virus sources, but only one of the species was easily inoculated by the green peach aphid, and the expression of such a differential was dependent upon the species of insect used (Sylvester and Simons, 1951). Similar examples can be deduced from other published data although they have not been interpreted as such (Kvíčala, 1948*b;* Severin and Tompkins, 1948*a*).

An explanation of vector specificity could be made independent of a mechanical transmission hypothesis, but when extended to cover the various phenomena known, it would probably involve assumption of pairs of inactivators, one fasting labile, and the other fasting stable. However, this would appear to be an unnecessary complication at the present time.

It appears necessary that the inactivator be more or less unaffected by fasting since in instances where vectors transmit with very low efficiencies, little effect of fasting can be demonstrated, and in those instances where slight gains in efficiency occur, it is only after relatively long fasting periods.

If transmission is entirely mechanical, as suggested by Bradley (1952), then a necessary corollary is that fasted insects feed more frequently without the immediate accompaniment of salivary secretion than nonfasted. Whether this is entirely supported by observation is questionable. The limited observations by Bradley (1952) are indicative, but in the writer's limited experience it has been extremely difficult to ascertain the moment when saliva begins to flow, and the insects appear to form the sheath as soon as possible after penetration through the cuticle and epidermal cell wall. It has been observed that the stylets frequently break through the sheath during its formation and moulding and then they are immediately withdrawn and more saliva is ejected with subsequent resumption of the moulding process. It may

be that during such break-through periods, virus is acquired. However, if it is hypothecated that transmission efficiency is dependent on compatibility within the saliva-virus-host plant cell combination then it must be assumed in the case of nontransmission that saliva accompanies every penetration, and consequently if virus pickup is independent of saliva it would seem that it might occur when the stylets are forced through the end of the sheath during the moulding process and that this might occur more frequently with fasted insects than with nonfasted. It is also possible that fasted insects ingest samples of plant sap sooner and more frequently than do nonfasted.

SUMMARY AND CONCLUSIONS

Additional work on the transmission of the *Brassica nigra* virus by the green peach aphid, *Myzus persicae* (Sulzer), to *Brassica juncea* seedling indicated the following:

1. Acquisition feedings allowed to terminate normally increased the probability of obtaining an infection over those which were terminated abnormally. This was not true in connection with test feedings.

2. Preliminary fasting before a short acquisition feeding increased the transmission efficiency of both the green peach aphid and the turnip aphid, *Rhopalosiphum pseudobrassicae* (Davis). The green peach aphid was the better vector, and a response to fasting was indicated within a 5-minute period. The beneficial effects of preliminary fasting were not noticeable with the turnip aphid until 4 hours later.

3. Five minutes of post-acquisition fasting decreased the level of infectivity in the green peach aphid. The data on the turnip aphid were too few for comparative purposes, but no gain in efficiency occurred as a result of any post-acquisition fasting interval tested.

4. Lowering the temperature (to 5°C) decreased the rate of loss of efficiency due to post fasting, and also the rate of gain in efficiency due to preliminary fasting.

5. Multiple 15-second acquisition feedings of four or less caused little increase in infectivity in green peach aphids. If five or more 15-second feedings were used, there was a tendency for gain in vector infectivity.

6. Serial transmission trials indicated that dispersal of virus charge by individuals was somewhat at random, and that multiple acquisition feedings, while increasing the number of infective individuals in a population, did not effectively increase the infectivity level within individuals.

7. An access time of 15 or more minutes decreased vector efficiency when compared with a 5-minute period. However, a 5-minute access period results in a greater number of infective individuals than a controlled 15-second watched-timed acquisition feeding. Increasing access time beyond 15 minutes was detrimental to vector efficiency.

8. Records kept on the activity of aphids during a 5-minute access period indicated that an average of three or four punctures was made, and that the majority of these were longer than 15 seconds. This increased feeding activity could account for some of the gain in transmission efficiency of insects which had had a 5-minute access period compared with those which had had a single controlled 15-second feeding.

9. Use of access periods varying in length from 5 minutes to 24 hours indicated that after 4 hours of feeding on a virus source plant few if any aphids were infective.

10. Trials comparing loss of virus during feeding and during fasting indicated that infective vectors feeding on a healthy plant lost virus more rapidly than those which were fasted. Feeding insects retained virus for a maximum of 30 minutes, while fasted insects retained infectivity for 3 hours.

11. Serial transmission trials designed to determine variations in virus charge among individual aphids indicated that the average charge per individual was approximately the same whether the insect fed for 15 seconds or for an access period of 5 minutes.

12. In tests designed to determine if retention of virus during post-acquisition fasting periods could be used to measure differences in virus charge among individuals, the results indicated that individuals which had had a 5-minute access period retained virus longer, with less percentage loss per test interval than those which had had either one 15-second acquisition feeding or five separate 15-second acquisition feedings. Thus there were indications that post-acquisition fasting might be used as a more critical test for determination of virus charge that that of serial transmission.

13. A review is presented of the available literature on the relation of anatomy and feeding of aphids, the acquisition, inoculation, and transmission threshold periods, preliminary and post-acquisition fasting, and length of acquisition feeding. Also presented are multiple stylet penetrations, serial transmission, virus charge, vector efficiency and specificity, virus retention, age and form of insects, numbers of insects, and variations due to temperature, light, humidity, host plants, and test plants, to the transmission of nonpersistent viruses by aphids, and a discussion of the various hypotheses concerning the mode of transmission, namely, mechanical or otherwise. An additional hypothesis, based upon the experimental data and a recombination of existing ideas, is offered. It is proposed that transmission of nonpersistent viruses by aphids is in essence mechanical, and that vector efficiency and specificity are due to compatibility factors which are dependent upon specific interactions among the viruses, the saliva of the aphids, and the host plant cells being inoculated.

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NOTE

Since the preparation of this publication, several contributions to the literature concerning the insect transmission of plant viruses have been made. These references, not included in the text, are as follows:

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